UDC: 63 ISSN 1450-8109

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE

Vol. 69, No. 2, 2024

Published by University of Belgrade
Faculty of Agriculture
Republic of Serbia

UDC: 63 ISSN 1450-8109

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE

Vol. 69, No. 2, 2024

Published by University of Belgrade
Faculty of Agriculture
Republic of Serbia

Journal of Agricultural Sciences (Belgrade) www.joas.agrif.bg.ac.rs
J. Agric. Sci (Bg).

PUBLISHED BY

University of Belgrade-Faculty of Agriculture
11081 Belgrade-Zemun, Nemanjina 6, PO Box 14, Serbia
Tel: + 381 11 4413-555/467; Fax: + 381 11 2193-659; E-mail: redakcija@agrif.bg.ac.rs
URL: http://www.agrif.bg.ac.rs/

DTP Service: Snežana Spirić

Printed by the Faculty of Agriculture, Belgrade-Zemun
Circulation: 100

Publishing is supported by the Ministry of Education, Science and Technological Development
of the Republic of Serbia, Belgrade

According to the opinion of the Ministry of Science of the Republic of Serbia
No. 413-00-1928/2001-01 dated November 6, 2001 this
Journal is exempt from general tax liability

Frequency: Four times per year

Abstracting and Indexing
CAB Abstracts, AGRICOLA, SCIndeks, EBSCO, Scopus, DOAJ

Number of institutions the Journal is exchanged with: 80

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE
Vol. 69, No. 2, 2024

C O N T E N T S

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE
Vol. 69, No. 2, 2024

S A D R Ž A J

Journal of Agricultural Sciences (Belgrade)
Vol. 69, No. 2, 2024
Pages 121-134

https://doi.org/10.2298/JAS2402121K
UDC: 633.11:631.547.1
547.52:631.547.1
Original scientific paper

EVALUATION OF THE EFFECTS OF SOME BENZIMIDAZOLE DERIVATIVES ON GERMINATION PARAMETERS OF WHEAT VARIETIES

Seda Külen[1*] and Sebla Dincer[2]

[1]Republic of Turkey Ministry of Agriculture and Forestry, Central Research Institute for Field Crops, Department of Quality and Technology, Ankara, Türkiye
[2]Ankara University, Department of Chemistry, Ankara, Türkiye

Abstract: This study aims to investigate the effect of some benzimidazole derivatives on the germination parameters of bread and durum wheat varieties. These derivatives contain both unsubstituted and substituted benzimidazole structures to determine the effect of the substituents on germination. Three different durum wheat varieties (Çeşit-1252, Eminbey, and Kızıltan-91) and three different bread wheat varieties (Demir 2000, Bayraktar 2000, and Tosunbey) were used in the study. First, 1*H*-benzimidazole (1) and 5-nitro-1*H*-benzimidazole (2) compounds were used to synthesize dinitro compounds (5,6-dnitro-1*H*-benzimidazole (3), 5,6-dinitro-2-methyl-1*H*-benzimidazole (4)) in order to investigate the effects of wheat on germination characteristics. Subsequently, these compounds were each reduced to nitroamine compounds (2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5), 5-nitro-1*H*-benzimidazole-6-amine (6)). Azo dyes (1-[(5-nitro-1*H*-benzimidazol-6-yl)diazenyl]naphthalene-2-ol (7), 1-[(2-methyl-5-nitro-1*H*-benzimidazol- 6-yl)diazenyl]naphthalene-2-ol (8)) and Schiff base compound (2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-yl)imino]methyl}phenol (9)) were synthesized from nitroamine compounds. Wheat samples whose germination parameters were to be investigated were treated with benzimidazole derivatives synthesized at a concentration of 10^{-6}M. Germination rates, coleoptile lengths, and root lengths of wheat varieties were measured on the 8th day of germination. The obtained results demonstrated that the efficiency of the benzimidazole compounds varied depending on the wheat varieties and germination parameters. 5-nitro-1*H*-benzimidazole-6-amine (6) had the greatest effect on germination rate while 1*H*-benzimidazole (1) had the greatest effect on root and coleoptile lengths in all varieties.

Key words: benzimidazole, wheat germination, chemical stimuli.

[*]Corresponding author: e-mail: seda.kulen@tarimorman.gov.tr

Introduction

Among the heterocyclic compounds, benzimidazoles have found a wide range of applications and their derivatives are employed in many different fields of research, particularly in agriculture and medicine. The structure of the benzimidazole compound is very similar to the structure of the purine bases, which are the cornerstones of the biological system. Due to their low toxicity, metal binding, and great stability, benzimidazole derivatives are one of the most significant study subjects (Reddy, 2010; Al-ebaisat, 2011; Tahlan et al., 2019).

From an agricultural point of view, there are many studies on the germination of wheat. In recent years, the inhibition or activation effects of chemicals on germination parameters and the enzymatic activity during germination have been investigated (Ansari & Lal, 2009; Ezzat, 2021; Gudasi et al., 2005; Tobina et al., 2014; Tot et al., 2018; Vasilache et al., 2014).

Enzymatic activity rises during germination. However, not every cereal experiences this in the same manner. The kind of enzyme, the grain, and the germination circumstances all play a role. In most cereals, the fourth day of germination marks the beginning of the peak activity of the enzymes (Guzmán-Ortiz et al., 2019). Phytic acid and other macromolecules and chemicals can be hydrolyzed thanks to germination. The ingredient in cereals with the most morphological alterations is starch. Some enzymes become more active and mobilized during germination. Factors influencing the activation time include the variety, temperature, and steeping period (Guzmán-Ortiz et al., 2019; Miransari and Smith, 2014). Typically, the addition of enzymes such as amylases, proteases, and xylanases modifies the rheology of the dough, the gas retention, and the softness of the crumb amylases (Guzmán-Ortiz et al., 2019). However, the timing of enzymatic activation varies depending on the type of cereal, growth circumstances, germination, and the availability of hormones such as gibberellins, which are produced in the grain embryos and stimulate the synthesis and production of enzymes, especially alpha-amylases (Guzmán-Ortiz et al., 2019).

The seed germination rate is one of the standard parameters for seed quality. There are many chemical stimuli whose effects on wheat germination have been investigated (Dhanda et al., 2004; Dyar & Shade, 1974; Gudasi et al., 2005; Risca et al., 2006; Vasilache et al., 2014). Some of the benzimidazole derivatives were investigated in the literature (Gudasi et al., 2005; Vasilache et al., 2014) in terms of germination parameters. However, to the best of our knowledge, the nitrobenzimidazole derivatives and the benzimidazole Schiff base compound used in this study have not yet been investigated for their effects on wheat germination.

The aim of this study is to evaluate the possible effects of nitrobenzimidazole derivatives and benzimidazole Schiff base compounds on wheat germination by examining the impact of benzimidazole derivatives on germination parameters

including germination rate, coleoptile length, and root length of three bread and three durum wheat varieties.

Material and Methods

Wheat varieties

Three different bread wheat varieties (Demir 2000, Bayraktar 2000, and Tosunbey) and three different durum wheat varieties (Çeşit-1252, Eminbey, and Kızıltan-91) obtained from the Ikizce field of the Ankara Field Crops Central Research Institute were used in this study. The wheat varieties were grown in 2018–2019 under rainfed conditions. The wheat varieties were selected based on their different grain qualities and features. Tosunbey is a hard white winter bread wheat, Demir 2000 is a red hard winter bread wheat and Bayraktar 2000 is a white medium-hard bread wheat. Tosunbey has strong gluten properties, Demir 2000 has medium-strong gluten properties and Bayraktar 2000 has medium-strong gluten properties. Eminbey is a durum wheat with high gluten quality, Çeşit-1252 has medium gluten quality and Kızıltan-91 has low gluten quality.

Germination method

Germination analyses were performed according to the modified ISTA method (ISTA, 2012). For germination analysis, 50 wheat seeds, which were firm, large and insect-free, were selected and placed on the special humidity papers in the germination pot and pre-soaked with 17 mL of the benzimidazole solutions prepared as 10^{-6} M in dilute methanol (1:20).

A filter paper wetted with 5 mL of the same solution was placed on it and the germination pot was placed in a 20ºC incubator (Stik-BI-250A) in the dark, at constant humidity and in random order. Each day, an additional 5 mL of benzimidazole solution was added to the germination pots. On the 8^{th} day of germination, the coleoptile and root lengths were measured on the randomly selected 10 germinated wheat grains from each germination pot, with three replications for each chemical application in each wheat variety.

Statistical analysis

The results obtained were analyzed using analysis of variance (ANOVA) using the JMP program, version 11.0.0 (SAS Institute Inc., 2013). When significant differences ($p<0.05$) were determined, the least significant difference (LSD) test was used to determine the differences among the means.

Synthesis of benzimidazole derivatives

1*H*-benzimidazole (1) and 5-nitro-1*H*-benzimidazole (2) were purchased from Sigma. The compounds (3-9) were synthesized by the authors and their characteristic

data: 5,6-Dinitro-1*H*-benzimidazole (3) and 5,6-dinitro-2-methyl-1*H*-benzimidazole (4) were obtained using the conventional nitration method of benzimidazoles (Patel et al., 2022). The preferential reduction of dinitrobenzimidazole derivatives gave 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) and 5-nitro-1*H*-benzimidazole-6-amine (6) (Dincer, 2002; Ozbay, 2010; Tsymliakov et al., 2023). 1-[(5-Nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol (7) was obtained as a red solid according to the standard methods of diazotization and coupling reactions using HNO_2 (Külen, 2021; Shafeeq et al., 2022), b.p. > 300 ºC, yield: 65%, FT-IR, (cm^{-1}) 3644, 3100, 2980, 2890, 1640, 1530, 1438, 1350); UV-VIS (nm, EtOH): 297, 497. 1-[(2-Methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol (8) was obtained as a red solid according to the method of (7) using naphthalene-2-ol, b.p. > 300 ºC, yield: 78%, FT-IR, (cm^{-1}) 3088, 1620, 1521, 1487, 1404, 1321, 1298; UV-VIS (nm, EtOH): 226, 298, 416, 502. 2-[(2-Methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol (9) was obtained as a yellow solid by the condensation reaction of 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) with salicylaldehyde (Gönülalan, 2011; Külen, 2021), m.p.: 325 °C, yield: 81%, element analysis, (%) $C_{15}H_{12}N_4O_3$: Found: C:58.73, H:4.07, N:18:42, Calculated: C:60.81, H:4.08, N:18.91; FT-IR, (cm^{-1}) 3103 cm^{-1}, 3054 cm^{-1}, 2979 cm^{-1}, 1623 cm^{-1}, 1605 cm^{-1}, 1573 cm^{-1}, 1516 cm^{-1}, 1352 cm^{-1}, ^{1}H-NMR (400 MHz, DMSO-d6), δ (ppm) 2.6 (3H, s), 6.8-7.2 (2H, m), 7.6 (1H, s), 8.2 (1H, s), 9.0 (1H, s), 12.5 (1H, s), 12.9 (1H, s). The chemical structures of the compounds used in the germination applications are given in Figure 1.

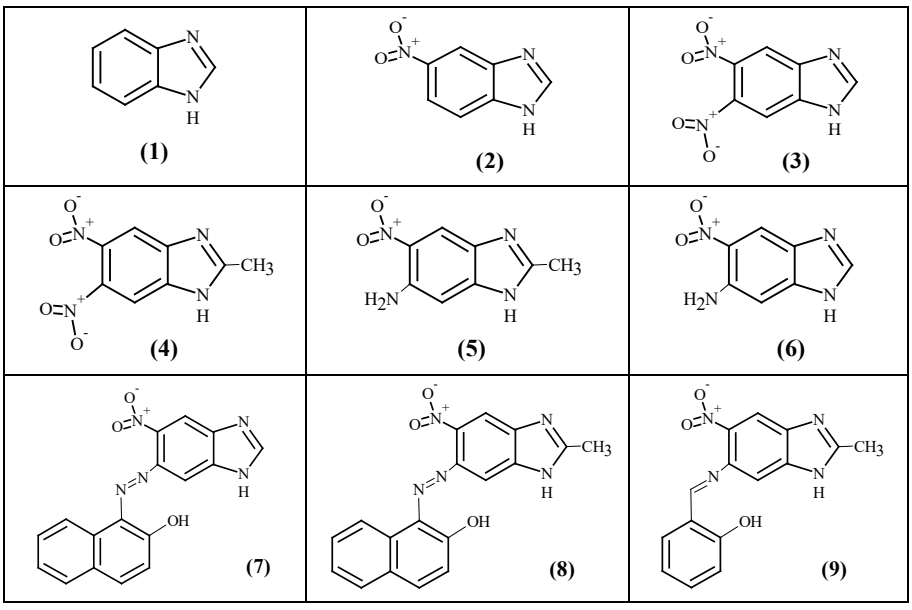

Figure 1. Compounds used in germination analysis.

Results and Discussion

Germination analysis

Coleoptile length (cm)

In monocotyledons, the coleoptile is a sheath that helps the shoot emerge through the soil crust. The maximum depth at which seeds can be sown depends on the length of the coleoptile. Therefore, genotypes with longer coleoptiles can be seeded deeper to avoid dry and hot conditions. Genotypes with shorter coleoptiles, on the other hand, may not sprout if sown too deeply, resulting in a weak stand and eventually causing production losses. Additionally, a rise in temperature has a negative impact on coleoptile length. Such variety-environmental interactions can therefore have a disastrous impact on crop productivity (Sidhu et al., 2020).

Table 1. Mean values of the coleoptile lengths measured in benzimidazole solutions in wheat varieties on the 8th day.

	Durum wheat			Bread wheat			
Application/ variety	Çeşit-1252	Eminbey	Kızıltan-91	Bayraktar 2000	Demir 2000	Tosunbey	Mean**
Control	7.77 ı-q*	6.40 ou	7.98 h-p	9.41 d-ı	9.57 c-ı	8.16 h-o	8.22 BC
(1)	9.71 b-h	8.36 g-m	10.60 a-e	9.16 d-j	10.96 a-d	7.99 h-p	9.46 A
(2)	6.68 m-t	4.10 v	8.21 h-o	8.40 f-m	10.15 b-g	8.12 h-o	7.61 CDE
(3)	8.82 e-k	8.10 h-o	10.25 b-f	8.62 f-l	6.47 n-u	6.89 l-t	8.19 BCD
(4)	7.74 ı-q	7.00 k-s	8.29 g-n	8.87 e-k	7.43 j-r	5.33 s-v	7.44 DE
(5)	6.80 l-t	6.62 m-t	6.17 p-u	7.85 h-q	9.25 d-j	6.03 q-u	7.12 E
(6)	5.45 s-v	5.44 s-v	4.62 uv	11.58 ab	12.45 a	11.31 abc	8.48 B
(9)	5.10 tuv	5.57 r-v	7.15 k-s	7.98 h-p	8.80 e-k	7.11 k-s	6.95 E
Mean***	7.26 B	6.45 C	7.91 B	8.98 A	9.39 A	7.62 B	

*Different lowercase letters indicate statistical differences between application/variety interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

The effects of the synthesized benzimidazole compounds on the coleoptile length of wheat varieties were evaluated and the results are given in Table 1. Better coleoptile length results were obtained by applying 5-nitro-1*H*-benzimidazole-6-amine (6) on bread wheat varieties. The mean values of the coleoptile length of Demir 2000, Bayraktar 2000, and Tosunbey varieties were 12.45, 11.58, and 11.31 cm, respectively. Besides, applying 1*H*-benzimidazole (1) had better results in

Demir 2000 and Kızıltan-91 varieties (10.96 and 10.60 cm) (Table 1). When comparing the effect of all chemical compounds on the mean coleoptile length of wheat varieties, Bayraktar 2000 and Demir 2000 obtained better results (8.98 and 9.39). Compounds (2), (4), (5), and (9) showed lower mean values of coleoptile length than the control sample, while the effects of compounds (3) and (6) on coleoptile length were statistically similar to the control sample (Table 1).

The effects of the compounds on the coleoptile length of wheat species were evaluated and it was observed that bread wheat varieties had a higher coleoptile length (8.66 cm) compared to durum wheat varieties (7.21 cm). Among the benzimidazole derivatives, the effect of 1*H*-benzimidazole (1) and 5-nitro-1*H*-benzimidazole-6-amine (6) on the coleoptile length (9.46 and 8.47 cm) was found to be better in the bread wheat varieties compared to durum wheat varieties (Table 2).

Table 2. Mean values of the coleoptile lengths measured in benzimidazole solutions in wheat species on the 8th day.

Application/species	Durum wheat	Bread wheat	Mean**
Control	7.38 efg*	9.05 bc	8.22 BC
(1)	9.56 b	9.37 b	9.46 A
(2)	6.33 ghı	8.89 bcd	7.61 BCD
(3)	9.06 bc	7.33 efg	8.19 BC
(4)	7.67 def	7.21 e-h	7.44 CD
(5)	6.53 fgh	7.71 def	7.12 D
(6)	5.17 ı	11.78 a	8.47 B
(9)	5.94 hı	7.96 cde	6.95 D
Mean***	7.21 B	8.66 A	

*Different lowercase letters indicate statistical differences between application/species interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

Root length (cm)

The development of a plant root, an essential organ for absorbing nutrients, is one of the key indicators of a plant's ability to withstand drought. From a physiological perspective, having a healthy root-to-shoot ratio helps plants cope with drought stress (Liu et al., 2015). In our study, the effects of the synthesized benzimidazole derivatives on the root length of wheat varieties were evaluated. The results are given in Table 3. It was observed that the application of unsubstituted benzimidazole (1) on Eminbey (13.78 cm); 5-nitro-1*H*-benzimidazole (2) on

Tosunbey (13.11 cm); 5,6-dinitro-1H-benzimidazole (3) on Eminbey (12.36 cm); 5,6-dinitro-2-methyl-1*H*-benzimidazole (4) on Bayraktar 2000 (11.53 cm); 5-nitro-1*H*-benzimidazole-6-amine (6) on Demir 2000 (12.92 cm); 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) on Eminbey (12.05 cm); 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-yl)imino]methyl}phenol (9) on Tosunbey (11.48 cm) had the highest root length values (Table 3). Among the benzimidazole derivatives, the effect of 1*H*-benzimidazole (1) on the root length was better. When considering the mean value of root length after applying all chemicals, Bayraktar-2000 (10.54 cm), Eminbey (10.81 cm) and Tosunbey (11.00 cm) were the most prominent varieties (Table 3).

Table 3. Mean values of the root lengths measured in benzimidazole solutions in wheat varieties on the 8th day.

	Durum wheat			Bread wheat			
Application/ variety	Çeşit-1252	Eminbey	Kızıltan 91	Bayraktar 2000	Demir 2000	Tosunbey	Mean**
Control	9.77 f-l*	11.44 a-h	10.42 e-k	11.83 a-g	10.45 e-k	10.43 e-k	10.72 BC
(1)	13.61 ab	13.78 a	11.41 b-ı	10.80 c-j	10.34 e-k	12.99 abc	12.15 A
(2)	8.39 k-l	9.79 f-l	12.28 a-e	11.51 a-h	11.13 c-j	13.11 abc	11.04 B
(3)	11.70 a-h	12.36 a-e	10.80 c-j	10.93 c-j	7.68 l-m	10.62 d-k	10.68 BC
(4)	9.45 h-l	10.58 d-k	9.88 f-l	11.53 a-h	9.73 f-l	9.69 g-l	10.15 BC
(5)	9.07 ı-l	12.05a-f	9.40 h-l	10.18 e-k	8.96 j-l	9.91f-l	9.93 C
(6)	5.62 mn	5.56 mn	4.71 n	7.62 lm	12.92 a-d	9.74 f-l	7.7 D
(9)	9.70 f-l	10.94 c-j	10.12 e-k	9.9 f-l	9.43 h-l	11.48a-h	10.26 BC
Mean***	9.67 D	10.81 AB	9.88 CD	10.54 AC	10.08 BCD	11.00 A	

*Different lowercase letters indicate statistical differences between application/variety interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole,; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

The effects of the compounds on the root length value of wheat species were evaluated and the results are given in Table 4. There was no significant difference between the mean root lengths of bread wheat and durum wheat. Among the benzimidazole derivatives, the application of 1*H*-benzimidazole (1) had the highest mean root length value (12.15 cm) (Table 4). The application of 5-nitro-1*H*-benzimidazole (2) on bread wheat (11.91 cm) and 5,6-dinitro-1*H*-benzimidazole (3) on durum wheat (11.62 cm) had the better root length results. The application of unsubstituted benzimidazole also had positive effects on the root length of both bread wheat (11.38 cm) and durum wheat (12.93 cm) varieties.

Table 4. Mean values of the root lengths measured in benzimidazole solutions in wheat species on the 8th day.

Application/species	Durum wheat	Bread wheat	Mean**
Control	10.54 b-e*	10.90 b-e	10.72 B
(1)	12.93 a	11.38 a-d	12.15 A
(2)	10.16 cde	11.91 ab	11.04 B
(3)	11.62 abc	9.75 e	10.68 B
(4)	9.97 de	10.32 cde	10.15 B
(5)	10.17 cde	9.68 e	9.93 B
(6)	5.30 f	10.10 cde	7.70 C
(9)	10.25 cde	10.27 cde	10.26 B
Mean^{ns}	10.12	10.54	

*Different lowercase letters indicate statistical differences between application/species interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);^{ns} There is no statistical difference between the species ($p<0.05$); (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino] methyl}phenol.

Germination rate (%)

In the germination analyses, germination was observed on the 8th day. The highest rate was generally noted in the control applications of Çeşit-1252 (97%), Eminbey (95%), Bayraktar 2000 (98%), Demir 2000 (97%) and Tosunbey (97%) (Table 5). The application of unsubstituted benzimidazole (1) to Bayraktar 2000 (97%), Demir 2000 (97%), Tosunbey (100%) cultivars, the application of 5-nitro-1*H*-benzimidazole (2) to Bayraktar 2000 (99%), Demir 2000 (98%), Tosunbey (99%) varieties, the application of 5,6-dinitro-1*H*-benzimidazole (3) to Eminbey (96%) Bayraktar 2000 (99%), Demir 2000 (95%), Tosunbey (97%) varieties, the application of 5-nitro-1*H*-benzimidazole-6-amine (6) to Çeşit-1252 (100%), Eminbey (98%), Kızıltan-91 (99%), Bayraktar 2000 (99%), Demir 2000 (100%) and Tosunbey (97%) varieties, the application of 2-methyl-5-nitro-1*H*-benzimidazole-6-amine (5) to Bayraktar 2000 (99%) and Demir 2000 (97%) varieties, the application of 5,6-dinitro-2-methyl-1*H*-benzimidazole (3) to Eminbey (96%), Bayraktar 2000 (99%) and Demir 2000 (96%) varieties, the application of 2-[(2-methyl-5-nitro-1*H*-benzimidazole-6-yl)imino]methyl}phenol (9) to Bayraktar-2000 (99%), Demir 2000 (97%) and Tosunbey (97%) varieties had the highest germination rate on the 8th day (Table 5).

Table 5. Mean values of the germination percentage values measured in benzimidazole solutions in wheat varieties on the 8th day.

	Durum wheat			Bread wheat			
Application/ variety	Çeşit-1252	Eminbey	Kızıltan-91	Bayraktar 2000	Demir 2000	Tosunbey	Mean**
Control	97 a-d*	95 a-f	93 c-h	98 abc	97 abc	97 a-d	96 B
(1)	89 h-k	87 ı-k	85 j-k	97 a-d	97 a-d	100 a	92 D
(2)	92 d-h	92 d-h	91 f-ı	99 a	98 abc	99 ab	95 BC
(3)	94 b-g	96 a-e	84 k	99 ab	95 a-f	97 a-d	94 CD
(4)	91 e-ı	96 a-e	89 hjk	99 ab	96 a-e	91 e-ı	94 CD
(5)	89 g-j	95 ae	91 e-ı	99 a	97 abc	93 c-h	94 BC
(6)	100 a	98 a-c	99 a	99 ab	100 a	97 abc	99 A
(9)	89 h-k	92 d-h	94 b-g	99 a	97 a-d	97 ad	95 BC
Mean***	93 C	94 C	91 D	99 A	97 AB	96 B	

*Different lowercase letters indicate statistical differences between application/variety interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

The 5-nitro-1*H*-benzimidazole-6-amine (6) had the highest germination percentage (99%) among the mean germination rates of the applied compounds. The bread wheat varieties Bayraktar 2000 (99%) and Demir 2000 (97%) had the highest mean germination rate (Table 5). The effects of the compounds on the germination rate of wheat species on the 8th day were evaluated and it was observed that the bread wheat varieties had a higher germination rate (97%) compared to the durum wheat varieties (92%) (Table 6).

When the interaction of the applied chemicals with the species was examined, it was found that the effect of all chemical applications on the germination rate of bread wheat was better than that of durum wheat. Among the benzimidazole derivatives, only 5-nitro-1*H*-benzimidazole-6-amine (6) had a high germination rate value (99%) in the durum wheat varieties (Table 6).

Table 6. Mean values of the germination percentage values measured in benzimidazole solutions in wheat species on the 8th day.

Application/species	Durum wheat	Bread wheat	Mean**
Control	95 bc*	97 ab	96 B
(1)	87 e	98 ab	92 D
(2)	92 d	99 a	95 BC
(3)	91 d	97 ab	94 BCD
(4)	92 cd	95 b	94 CD
(5)	92 cd	97 ab	94 BCD
(6)	99 a	99 a	99 A
(9)	92 d	98 ab	95 BCD
Mean***	92 B	97 A	

*Different lowercase letters indicate statistical differences between application/species interactions, ($p<0.05$);**Different capital letters in the same column indicate statistical differences between applications ($p<0.05$);*** Capital letters in the same row indicate the statistical difference between varieties ($p<0.05$), according to the LSD test (1) 1*H*-benzimidazole; (2) 5-nitro-1*H*-benzimidazole; (3) 5,6-dinitro-1*H*-benzimidazole; (4) 5,6-dinitro-2-methyl-1*H*-benzimidazole; (5) 2-methyl-5-nitro-1*H*-benzimidazole-6-amine; (6) 5-nitro-1*H*-benzimidazole-6-amine; (7) 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol; (8) 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol; (9) 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol.

It was observed that bread wheat (97%) had a higher germination rate than durum wheat (92%) on the 8th day of germination. Another result obtained by evaluating the analysis data showed that Bayraktar 2000 (99%) and Demir 2000 (97%) were the bread wheat varieties with the best germination. Analyzing the impact of chemical applications on the coleoptile length of the varieties, it was concluded that the application of 5-nitro-1*H*-benzimidazole-6-amine (6) to Demir 2000 (12.45 cm), Bayraktar 2000 (11.58 cm) and Tosunbey (11.31 cm) varieties and the application of 1*H*-benzimidazole (1) to Demir 2000 (10.96 cm) and Kızıltan-91 (10.60 cm) varieties gave the highest coleoptile length values. While the highest result was obtained with 1*H*-benzimidazole (1) in the applied compounds, Bayraktar 2000 and Demir 2000 varieties were the most prominent among the varieties.

The viability of the grains for seeding, and the size and mass of the cereal grains can be used to explain the variance in germination rates between the various cereal grains (Kaur and Gill, 2020). On the other hand, the results obtained can be evaluated in terms of the chemical structures of the applied compounds (Dincer, 1992; Hameed et al., 2016, 2019). According to the study of Vasilache et al. (2014), the existence of methoxy or ethoxy radicals in the molecules is connected to the biological effects of imidazole derivatives, which are thought to have an auxin-like effect by stimulating cell elongation. Some authors have shown that imidazolium-based ionic liquids are toxic to barley and wheat seedlings during the early stages of growth. The most toxic ionic liquid is the one with the longest alkyl

substituent on the imidazolium ring (Bubalo et al., 2014; Chen et al., 2016). Tot et al. (2018) studied how ionic liquids based on imidazolium affected the germination and growth of wheat and barley. The observed effects of the chemicals on wheat and barley germination strongly imply that the total toxicity of the ionic liquids was influenced by the addition of oxygen as a hydroxyl group in the alkyl substituent. These results are consistent with the results of the present study, according to which the Schiff base 2-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)imino]methyl}phenol (9), which has the -OH group, had no positive influence on the length of the coleoptile or the root. The chemical 5-nitro-1*H*-benzimidazole-6-amine (6), which contains a free -NH_2 group, was found to have a stronger impact on the germination rate in this study. Derivatives of benzimidazoles, which have a diazo group in their structure, were found to inhibit germination. Since the germination properties of these chemicals have never been investigated before, the results of the present study are significant.

Conclusion

The objective of this study was to investigate the effects of benzimidazole derivatives with different substituents and different structures on the germination parameters of wheat. These substituted benzimidazole derivatives have not yet been studied in the literature with regard to germination analysis.

The results indicated that the benzimidazole compounds had different effects on the germination parameters of the different species (bread wheat and durum wheat). Of all the benzimidazole derivatives, 5-nitro-1*H*-benzimidazole-6-amine (6) exhibited a high value for germination rate in durum wheat varieties. Besides that, the outcomes can also be assessed in terms of the chemical structures of the substances used. A molecule with a free -NH_2 group, 5-nitro-1*H*-benzimidazole-6-amine (6), had the highest germination percentage on the 8^{th} day. However, at a concentration of 10^{-6} M, the azo benzimidazole derivatives, 1-[(5-nitro-1*H*-benzimidazole-6-yl)diazenyl]naphthalene-2-ol (7) and 1-[(2-methyl-5-nitro-1*H*-benzimidazol-6-il)diazenyl]naphthalene-2-ol (8), with the -N=N- group in the structure, inhibited the wheat germination. If we consider the result that diazo compounds inhibit germination, the possibilities of using these compounds and their derivatives as herbicides should be investigated. The results of the present study suggest that some benzimidazole derivatives could be used to enhance the germination rate, coleoptile, and root lengths of wheat.

Further studies with a higher number of wheat genotypes containing different species are needed to confirm these findings.

References

Al-ebaisat, H.S. (2011). Synthesis and Biological Activities of Some Benzimidazoles Derivatives. *Journal of Applied Sciences and Environmental Management, 15*, 451-454.

Ansari, K.F., & Lal, C. (2009). European Journal of Medicinal Chemistry Synthesis, physicochemical properties and antimicrobial activity of some new benzimidazole derivatives. *European Journal of Medicinal Chemistry*, 44, 4028-4033.

Bubalo, M.C., Hanousek, K., Radošević, K., Srček, G.V., Jakovljević, T., & Redovniković, I.R. (2014). Imidiazolium based ionic liquids: effects of different anions and alkyl chains lengths on the barley seedlings. *Ecotoxicology and Environmental Safety, 101,* 116-123.

Chen, Z., Dai, B., Zhang, W., Guan, W., Liu, N., & Liu, K. (2016). Study on the growth of wheat seedlings under acetic acid ionic liquids [Cnmim][OAc] (n ¼ = 2, 4, 6). *RSC Advances, 6,* 96908-96913.

Dhanda, S.S., Sethi, G.S., & Behl, R.K. (2004). Indices of Drought Tolerance in Wheat Genotypes at Early Stages of Plant Growth. *Journal of Agronomy and Crop Science*, *190*, 6-12.

Dincer, S. (1992). Synthesis of new plant growth hormones and investigation of their effects. Ankara Üniversitesi.

Dincer, S. (2002). The preferential reduction of 4,6 (5,7)-dinitro and 5,6-dinitro benzimidazoles. *Dyes and Pigments*, *53*, 263-266.

Dyar, J.J., & Shade, J. (1974). The Influence of Benzimidazole on the Gametophyte of Thelypteris felix-mas. *Plant physiology,* 53, 666-668.

Ezzat, S.E. (2021). Effect of Thiamine and Biotin Vitamins on Growth and Yield of Smooth Wheat Triticum aestivum L. *Annals of the Romanian Society for Cell Biology*, *25*, 5305-5313.

Gönülalan, G. (2011). Synthesis and Structure Investigation of New Benzimidazole Schiff Bases. Ankara University.

Gudasi, K.B., Shenoy, R.V, Vadavi, R.S., Patil, M.S., Patil, S.A., Hanchinal, R.R., & Lohithaswa, H. (2005). Lanthanide (III) and Yttrium (III) Complexes of Benzimidazole-2-Acetic Acid: Synthesis, Characterisation and Effect of La (III) Complex on Germination of Wheat. *Bioinorganic Chemistry and Applications*, *2006*, 1-8.

Guzmán-Ortiz, F.A., Castro-Rosas, J., Gómez-Aldapa, C.A., Mora-Escobedo, R., Rojas-León, A., Rodríguez-Marín, M.L., Falfán-Cortés, R.N., & Román-Gutiérrez, A.D. (2019). Enzyme activity during germination of different cereals: A review. *Food Reviews International*, *35*, 177-200.

Hameed, A., Hameed, A., Ahmad, M., Saqlain, T., Farooq, T., Aslam, S., & Kanwal, A. (2016). Triazole priming improves germination potential, antioxidant levels and reduces oxidative damage in wheat seeds. *Philippine Agricultural Scientist*, *99*, 164-170.

Hameed, A., Hameed, A., Farooq, T., Noreen, R., Javed, S., Batool,S., & Ahmad, M. (2019). Evaluation of structurally different benzimidazoles as priming agents, plant defence activators and growth enhancers in wheat. *BMC Chemistry*, *13*, 1-11.

ISTA (2012). *International Rules for Seed Testing*. Bassersdorf, Switzerland: International Seed Testing Association.

Kaur, H., & Gill, B.S. (2020). Comparative evaluation of physicochemical, nutritional and molecular interactions of flours from different cereals as affected by germination duration. *Journal of Food Measurement and Characterization*, *14*, 1147-1157.

Külen, S. (2021). Synthesis and Characterization of Novel Transition Metal Complexes of Some 2-Substituted Benzimidazole Derivatives and Investigation Their Influence on Whea*t* Germination Parameters. Ankara University.

Liu, M., Li, M., Liu, K., & Sui, N. (2015). Effects of Drought Stress on Seed Germination and Seedling Growth of Different Maize Varieties. *Journal of Agricultural Science*, *7*, 231-240.

Patel, D.M., Patel, N.K., Narolkar, S., & Mishra A. (2022). In silico Studies and Assessment of Antimicrobial Activities for Synthesised Nitrobenzimidazole Derivatives. *Oriental Journal of Chemistry*, *38*, 432-438.

Miransari, M., & Smith, D.L. (2014). Plant hormones and seed germination. *Environmental and Experimental Botany*, *99*, 110-121.

Ozbay, S. (2010). *The New Synthesis of Some Aminobenzimidazoles.* Ankara University.

Reddy, B.A. (2010). Synthesis and Characterization of Some Benzimidazole Derivatives Using as Anti-hypertensive Agents. *Asian Journal of Pharmaceutical Research and Health Care*, *2*, 103-113.

Risca, I.M., Zbancioc, G., Moldoveanu, C., Drochioiu, G., & Mangalagiu, I. (2006). Effect of some new monoquaternary salts of diazine on germination and seedling growth of Norway spruce (Picea abies (L.) Karsten). *Romanian biotechnological letters, 11*, 2563-2568.

Shafeeq, A.A., Hamandi, Z.M.A., Mohammed, M.H., & Al-Shamma, G. (2022). Synthesis, Characterization, and Antimicrobial Evaluation for New Azo-linked Derivative of Heterocyclic Compounds. *Journal of Contemporary Medical Sciences*, *8*, 51-58.

Sidhu, J.S., Singh, D., Gill, H.S., Brar, N.K., Qiu, Y., Halder, J., & Sehgal, S.K. (2020). Genome-Wide Association Study Uncovers Novel Genomic Regions Associated With Coleoptile Length in Hard Winter Wheat. *Frontiers in Genetics*, *10*, 1-13.

Tahlan, S., Kumar, S., Kakkar, S., & Narasimhan, B. (2019). Benzimidazole scaffolds as promising antiproliferative agents: a review. *BMC Chemistry*, 1-16.

Tobina, H., Choi, J.H., Asai, T., Kiriiwa, Y., Asakawa, T., Kan, T., & Kawagishi, H. (2014). 2-Azahypoxanthine and imidazole-4-carboxamide produced by the fairy-ring-forming fungus increase wheat yield. *Field Crops Research*, *162*, 6-11.

Tot, A., Vraneš, M., Maksimović, I., Putnik-Delić, M., Daničić, M., Belić, & S., Gadžurić, S. (2018). The effect of imidazolium based ionic liquids on wheat and barley germination and growth: Influence of length and oxygen functionalization of alkyl side chain. *Ecotoxicology and Environmental Safety*, *147*, 401-406.

Tsymliakov, M.D., Maksutova, A.I., Bezsonova, E.N., Zakharova, D.V., Grishin, Y.K., Tafeenko, V.A., & Lozinskaya, N.A. (2023). Sodium dithionite in the regioselective reduction of the ortho-positioned nitro group in 1-R-2,4-dinitrobenzenes. *Mendeleev Communications*, *33*, 121-123.

Vasilache, V., Moldoveanu, C., Fartais, L., & Risca, I. (2014). Effect of Some New Imidazole Derivatives on Wheat *(Triticum Aestivum*) Germination. *Revista de Chimie (Bucharest)*, *65*, 177-180.

Received: July 20, 2023
Accepted: March 29, 2024

PROCENA UTICAJA NEKIH DERIVATA BENZIMIDAZOLA NA PARAMETRE KLIJANJA SEMENA PŠENICE

Seda Külen[1*] i Sebla Dincer[2]

[1]Republic of Turkey Ministry of Agriculture and Forestry, Central Research Institute for Field Crops, Department of Quality and Technology, Ankara, Türkiye
[2]Ankara University, Department of Chemistry, Ankara, Türkiye

R e z i m e

Ovo istraživanje ima za cilj da istraži uticaj nekih derivata benzimidazola na parametre klijanja semena hlebne i durum pšenice. Ovi derivati sadrže nesupstituisane i supstituisane benzimidazolske strukture kako bi se utvrdio uticaj supstituenata na klijanje semena. U istraživanju su korišćene tri različite sorte durum pšenice (Çeşit-1252, Eminbey i Kızıltan-91) i tri različite sorte hlebne pšenice (Demir 2000, Bayraktar 2000 i Tosunbey). Prvo su korišćena jedinjenja 1*H*-benzimidazol (1) i 5-nitro-1*H*-benzimidazol (2) za sintezu dinitro jedinjenja (5,6-dinitro-1*H*-benzimidazol (3), 5,6-dinitro-2-metil-1*H*-benzimidazol (4)) kako bi se utvrdila reakcija sorti na tretman. Nakon toga, svako od ovih jedninjena je redukovano na nitroaminska jedinjenja (2-metil-5-nitro-1*H*-benzimidazol-6-amin (5), 5-nitro-1*H*-benzimidazol-6-amin (6)). Azo boje (1-[(5-nitro-1*H*-benzimidazol-6-il)diazenil]naftalen-2-ol (7), 1-[(2-metil-5-nitro-1*H*-benzimidazol-6-il)diazenil]naftalen-2-ol (8)) i Šifovo bazno jedinjenje (2-[(2-metil-5-nitro-1*H*-benzimidazol-6-il)imino]metil)fenol (9)) sintetizovani su iz nitroaminskih jedninjenja. Seme svih sorti pšenice tretirano je derivatima benzimidazola sintetizovanim u koncentraciji 10^{-6} M. Stope klijanja, dužine koleoptila i dužine klijanca merene su osmog dana klijanja. Dobijeni rezultati su pokazali da efikasnost benzimidazolskih jedinjenja varira u zavisnosti od sorti pšenice i parametara klijanja. Najveći uticaj na stopu klijanja imao je 5-nitro-1*H*-benzimidazol-6-amin (6), dok je 1*H*-benzimidazol (1) imao najveći uticaj na dužinu korena i koleoptila kod svih sorti.

Ključne reči: benzimidazol, klijanje pšenice, hemijski stimulusi.

Primljeno: 20. jula 2023.
Odobreno: 29. marta 2024.

[*]Autor za kontakt: e-mail: seda.kulen@tarimorman.gov.tr

Journal of Agricultural Sciences (Belgrade)
Vol. 69, No. 2, 2024
Pages 135-154

https://doi.org/10.2298/JAS2402135A
UDC: 582.685.2:575.2
582.685.2:631.527
Original scientific paper

PHENOTYPIC VARIATION AND SIMULTANEOUS SELECTION OF NUMBER OF LEAVES/PLANT AND SEED MASS IN JUTE MALLOW (*CORCHORUS OLITORIUS*)

Adekoya M. Adejoke[1*], Adeniji O. Taiwo[2], Ekanem U. Okon[3], Badmus A. Adeshile[2], Peter J. Melangu[4] and Olosunde O. Busayo[2]

[1]Department of Plant Science and Biotechnology, Federal University, Oye Ekiti, Nigeria
[2]Department of Crop Science and Horticulture,
Federal University Oye Ekiti, Ekiti State, Nigeria
[3]International Institute of Tropical Agriculture, Ibadan, Nigeria
[4]Adamawa State University, Mubi, Nigeria

Abstract: *Corchorus olitorius* is a leafy vegetable cultivated for the mucilage in its leaves. Leaf greenness, leaf number, leaf length, and leaf width are popular market traits for this vegetable. Little is known about the direct and indirect contribution of traits to leaf number and seed yield. Forty-two accessions were evaluated in a randomized complete block design with four replications during the 2017 and 2018 cropping seasons. The findings showed that accessions 25, 19, and 28 performed best for leaf length, accessions 31, 22, and 23 for the number of leaves/plant, accessions 4, 18, and 27 for the number of seeds/capsule and accessions 8, 11, and 7 for seed mass. The seed mass was positively related to leaf length, leaf width, and plant height at maturity, the number of seeds/capsule, the number of seeds/capsule and 100-seed mass. The number of leaves/plant was influenced by leaf length, leaf width, and branch length. The path analysis for seed mass showed that the number of branches/plant, seed mass/capsule, the number of seeds/capsule and capsule mass made a large contribution to seed yield. The indirect contribution of traits to the number of leaves/plant was small compared to the direct effect. The leaf length had the largest direct effect on the number of leaves/plant with its largest indirect effect by reducing seed mass. The direct contribution of leaf length to the number of leaves/plant was masked by the phenotypic expression of petal width. The number of branches/plant is a reliable index of seed yield improvement. Hybridization among the best-performing accessions for leaf number, leaf chlorophyll and seed yield will produce new varieties through selection.

Key words: jute mallow, phenotypic variation, character correlation, selection index, yield improvement.

[*]Corresponding author: e-mail: munawarm11@gmail.com

Introduction

Corchorus olitorius [L.] (jute mallow) is a shrub species in the family Malvaceae. Its origin is traced back to Africa, Asia and the Indo-Burmes region (Anonymous, 2008). Jute mallow is an underutilized leaf vegetable in sub-Saharan Africa. It is a warm-weather crop with a C_4 cycle of photosynthesis. A temperature between 25 and 32^{o}C, rainfall of 600 to 2000 mm annually, and a day length of 12.5 hours produced a high fresh and dry leaf mass at culling (Grubben and Denton, 2004). Jute mallow is grown primarily in Africa, the Middle East, and Asia for its fresh leaves, and to a lesser extent for the fibers in the stem. The vegetable serves as an alternative food source for people in rural and urban areas. The leaves are rich in protein, thiamine, riboflavin, niacin, folate, and dietary fiber, beta-carotene, iron, calcium, and vitamin C (Grubben and Denton, 2004; Lewu and Mavengahama, 2010). The mucilage in the fresh or dried leaves (Whitlock et al., 2003; Ghosh et al., 2017; Ngomuo et al., 2017) enhances the mastication of starchy foods made from cassava flour, pounded boiled cassava and cassava granules, yam flour, maize, sorghum, and rice flour (Benor et al., 2012). Jute mallow is cultivated under a short harvest cycle. The young shoots are culled by uprooting 4 to 5 weeks after sowing. The number of leaves, leaf size, color and taste are market traits. The growers broadcast the seeds on the beds, which increases the demand for seeds. Farmers have consistently provided seeds for sowing. The inadequate supply of quality seeds due to the short cycle of culling is a major setback for sustainable production (Opabode and Adebooye, 2005).

The foliage yield of jute mallow is low, and there are only a few varieties with high leaf and seed yields. This is due to inadequate knowledge of the variability and interdependence of leaf and seed yield and the influence of the environment, which is important for the selection and development of high-yielding varieties. To enhance sustainable production, research needs to focus on the differences between accessions, the direct and indirect contribution of traits to the number of leaves per plant at culling, and high seed yield at maturity. This will ensure simultaneous improvement and increase the efficiency of selection. The correlation coefficient measures the mutual association between the traits. Path coefficient analysis is used to determine the relative contribution of independent traits directly to a dependent trait, and indirectly through other traits to increase selection efficiency. Crop growth and seed development patterns are controlled by genetic and environmental factors. The ability of a vegetable to provide leaves and seeds in different environments is a function of the response of traits to environmental attributes. There is limited information on the direct and indirect contribution of traits to the number of leaves/plant and seed mass, which is important for the selection and development of new varieties. The objectives of this study were 1) to estimate the extent of variation among the jute mallow accessions for agronomic and leaf yield-

related traits, 2) to determine the association between traits, and 3) to evaluate the extent of direct and indirect contribution of traits to the number of leaves/plant and seed mass.

Material and Methods

Sources of the accessions and experimental design

Forty-two accessions (Table 1) of jute mallow were evaluated at the Teaching and Research Farm, Federal University Oye Ekiti, Ikole Campus (longitude 7° 47′N and latitude 5°31′E, and 555 m above sea level) between August and December in 2017 and 2018. The research location has a bimodal rainfall pattern, i.e., the first rainfall season starts from April to July and from October to November.

Table 1. Forty-two accessions of jute mallow evaluated in Nigeria during 2017 and 2018.

Accession code	Source	Accessions code	Source
Acc 1	NaCGRAB[a]	Acc 23	FUOYE
Acc 2	NaCGRAB	Acc 25	FUOYE
Acc 3	NaCGRAB	Acc 26	FUOYE
Acc 4	NaCGRAB	Acc 27	FUOYE
Acc 5	NaCGRAB	Acc 28	FUOYE
Acc 6	NaCGRAB	Acc 29	FUOYE
Acc 7	NaCGRAB	Acc 30	FUOYE
Acc 8	NaCGRAB	Acc 31	FUOYE
Acc 9	FUOYE[b]	Acc 32	FUOYE
Acc 10	FUOYE	Acc 33	FUOYE
Acc 11	FUOYE	Acc 34	FUOYE
Acc 12	FUOYE	Acc 35	FUOYE
Acc 13	FUOYE	Acc 36	FUOYE
Acc 14	FUOYE	Acc 37	FUOYE
Acc 15	FUOYE	Acc 38	FUOYE
Acc 16	FUOYE	Acc 39	FUOYE
Acc 17	FUOYE	Acc 40	FUOYE
Acc 18	NaCGRAB	Acc 41	FUOYE
Acc 19	NaCGRAB	Acc 42	FUOYE
Acc 20	NaCGRAB	Acc 43	FUOYE
Acc 21	NaCGRAB		
Acc 22	NaCGRAB		

[a]Genetic Resources Unit, National Center of Genetic Resources and Biotechnology, Nigeria. [b]Crop Breeding Unit, Department of Crop Science and Horticulture, Federal University Oye Ekiti, Nigeria.

The rainfalls were abundant (700–1000 mm) with an annual temperature of 28°C and high humidity. Derived guinea savannah was the main vegetation. Before soil preparation, the soil of the experimental site had a pH (in H_2O, 1:1) of 5.7, OM content (%) of 0.82, N (%) content of 0.08, available phosphorus (Bray method) of 219.33, exchangeable Mg content (C mol kg^{-1}) of 0.19, exchangeable K content (C $molkg^{-1}$) of 0.48, exchangeable Na content (C mol kg^{-1}) of 0.07, exchangeable Ca content (C mol kg^{-1}) of 2.93, ECEC of 3.68, and Zn (C mol kg^{-1}) of 1.12, copper (Cu) (C mol kg^{-1}) of 1.01, Mn (C mol kg^{-1}) of 107.7, Fe (C mol kg^{-1}) of 180.07, the sand content of 68%, the silt content of 20%, the clay content of 11%, and the textural class – sandy loam.

Before sowing, seed dormancy was broken by hot and cold water treatment. The air-dried seeds were sown in multi-pot seedling trays, each tray holding 60 seedlings. Water was applied to the trays using a hand sprayer. The seedling trays were covered with a black nylon sheet and placed in a germination room at 22°C for 72 hours. The trays were then returned to the nursery shed, and the developing seedlings were watered once or twice a day for up to 4 weeks. One week before transplanting, 1 g of urea was dissolved in 1 l of water and applied to the seedlings using a hand sprayer. The field trial was conducted according to a randomized complete block design with four replications. Each plot was planted in two rows with a length of 7 m and a distance of 1 m between the rows. At 4 weeks old, vigorous seedlings were transplanted with the soil ball at a distance of 0.75 m between the plants on the side of the ridges 1 m apart. Two weeks after transplanting, 30 g of a fungicide (Ridomill WP) was dissolved in 20 l of water and the plants were sprayed. Weeds were removed manually using hoes at 6, 8, and 12 weeks after transplanting (WAT). An insecticide, Karate EC (20 ml in 20 liters of water), was sprayed at 5 WAT to reduce insect infestations and insect-borne diseases. A foliar application of organic fertilizer, Super Gro fertilizer (ethoxylated, alkylphenol), and polysiloxane, (manufactured by GNLD International, NAFDAC No. A5-0590) was applied at a rate of 100 ml in 10 liters of water at six and eight WAT. Physiological and reproductive traits were monitored until culling (Table 2) following the descriptor list published for *Corchorus olitorius* (Anonymous, 2008). The leaf chlorophyll content was measured at 5 weeks on 30 randomly sampled leaves in three replications using a chlorophyll meter machine (CCM-200 *PLUS*, Opti-Sciences, United States of America).

Table 2. Traits and descriptions of jute mallow accessions evaluated in Nigeria between 2017 and 2018.

Trait	Description
Days to 50% flowering (d)	Number of days from sowing to the opening in 50% of the plants on each plot.
Number of leaves/plant at flowering	The number of leaves/plant was counted on 10 randomly selected plants per accession at flowering.
Plant height at maturity (m)	Measured from the ground level to the tip of the highest point on 10 plants randomly selected at maturity.
Leaf length at flowering (cm)	Measured with a meter rule at the longest point on 10 leaves randomly selected at 50% flowering.
Leaf width at flowering (cm)	Measured with a meter rule at the widest point on 10 leaves randomly selected at 50% flowering.
Branch length at flowering (cm)	Branch length at 50% flowering was measured with a meter rule from the point of attachment to the plant to the other end.
Leaf chlorophyll	Determined on 10 leaves randomly selected/accession using a chlorophyll meter machine.
Number of branches/plant	Counted on five plants randomly selected per accession at maturity.
Petal length (cm)	Determined on the longest point on 10 petals randomly selected with a meter rule at flowering.
Petal width (cm)	Measured on the widest point on 10 petals randomly selected per accession with a meter rule at flowering.
Sepal length (cm)	Measured at the longest point on 10 sepals randomly selected at flowering with a meter rule.
Sepal width (cm)	Measured at the widest point on 10 sepals randomly selected at flowering with a meter rule.
Stamen height (cm)	Measured at the highest point on 10 stamens randomly selected at flowering.
Pistil height (cm)	Measured at the highest point on five randomly selected pistils at flowering.
Flower size	Small flower size = 1, large flower size = 2
Number of capsules per plant	The average number of capsules per plant on 5 plants randomly harvested at culling.
Capsule length (cm)	The average length of 10 capsules per plant randomly taken from 5 plants at culling.
Capsule mass (g)	Dried capsules harvested from five randomly sampled plants were weighed on a sensitive weighing balance.
Number of loculus/capsule	The average number of loculi/capsule taken from 5 randomly selected capsules/plant.
100-seed mass (g)	Average 100-seed mass measured on a sensitive weighing balance.
Number of seeds per capsule	The average number of seeds/capsule from 10 capsules per plant randomly selected from 5 plants.
Seed mass/capsule (g)	The number of seeds/capsule at culling were air-dried, at constant weight, the average weight of seed (g) from 10 capsules was determined from 5 randomly selected plants.
Seed mass/plant (kg)	The number of harvested seeds/plant were air-dried and weighed on a sensitive weigh balance.

The agronomic traits were averaged over the years and the homogeneity of error variance was tested. The performance of the accessions was tested over years. With insignificant accession × year interaction for most traits, the mean values showed little response to environmental factors. Subsequently, the accession means were used to perform the combined analysis of variance (ANOVA) for 44 accessions over years using the general linear model (PROC-GLM procedure of SAS, 2002) to partition the total variation into components due to genotype (G), year (Y), and G x Y interaction effects. The genotype was treated as a fixed effect and the year as a random effect. The multiple comparisons of the main effect were performed by the Tukey's HSD test. The Pearson's correlation coefficient between traits was determined using PROC CORR (SAS, 2012). The path coefficient analysis described by Wright (1921) and Dewey and Lu (1959) was used to determine the direct and indirect effect of independent traits on leaf number and seed yield (response variables). Other traits with positive correlation coefficients were considered as causal variables.

Results and Discussion

Variation in vegetative, reproductive, and seed yield traits

Significant ($P \leq 0.01$) mean squares were recorded for leaf chlorophyll, leaf length, leaf width, the number of leaves per plant, plant height at flowering, branch length at maturity, the number of branches per plant and plant height at maturity, petal length and width, pistil height, days to 50% flowering, flower size, capsule length, the number of capsules per plant, capsule mass, the number of seeds per capsule, seed mass and 100-seed mass (Table 3). This is consistent with the genetic constitution of the accessions and the influence of the environment. The findings are similar to previous reports by Fasinmirin and Olufayo (2009), Nwangburuka and Denton (2012) and Adebo et al. (2015), who noted differences among jute mallow accessions in terms of leaf morphology, vegetative, reproductive, capsule and seed yield traits. In contrast, low phenotypic variability in leaf length, leaf width, and the number of leaves/plant was found in jute mallow accessions from East Africa (Ngomuo et al., 2017). The year effect significantly ($P \leq 0.01$) influenced leaf width, the number of leaves per plant, plant height at flowering, plant height at maturity, petal length, pistil height, capsule length, and capsule weight, the number of seeds per capsule, and 100-seed mass. Mean squares of the genotype × year interaction were insignificant ($P \geq 0.05$) for all traits except for leaf length, number of leaves/plant, sepal length, stamen height and capsule length. The genotype accounted for a larger proportion of the total variation in all traits compared to the genotype × year interaction and year effects. The metric traits (plant height at flowering, number of seeds/capsule, 100-seed mass and capsule length) were responsive to precipitation, temperature, humidity, and soil factors

(year effect). This indicates that the year effect is important in defining phenotypic variability. This trend of observation could change if this evaluation were carried out elsewhere. Insignificant accession by year interaction for the traits implies that the genotypes were irresponsive to environmental factors, suggesting a high predictability of performance (Table 3).

Table 3a. Combined analysis of variance for vegetative traits in jute mallow accessions during 2017 and 2018.

Source of variation	df	Leaf chlorophyll	Leaf length (cm)	Leaf width (cm)	Number of leaves/plant	Plant height at flowering (cm)	Branch length at maturity (cm)	Number of branches	Plant height at maturity (cm)
Genotype	41	95.06**	47.55**	12.28**	4700.20**	208.91*	4520.84**	1238.88**	3475.68**
Rep (year)	5	1.03	3.50	0.76	16.10	165.90*	526.27	49.53	216.39
Genotype × year	83	1.99	1.92	1.83**	63.41**	26.53	483.47	29.77	33.73
Error	165	14.65	2.08	0.46	418.00	89.28	563.83	53.62	243.30
Mean		46.10	9.06	4.44	29.05	50.23	99.38	21.88	135.40

*, **0.05 and 0.01% levels of probability.

Table 3b. Combined analysis of variance for flower metric traits in jute mallow accessions during 2017 and 2018.

Source of variation	Df	Petal length (cm)	Petal width (cm)	Pistil height (cm)	Days to 50% flowering	Flower size	Sepal length	Sepal width	Stamen height
Genotype	41	0.42**	0.06**	0.18**	307.60**	4.34**	0.14**	0.02**	0.19**
Replicate (year)	5	0.01	0.002	0.001	356.11	0.02	0.0001	0.002	0.003
Genotype × year	83	0.04	0.006	0.001	33.73	0.00008	0.002*	0.005	0.01*
Error	165	0.01	0.002	0.001	24.40	0.04	0.009	0.007	0.74

*, **0.05 and 0.01% levels of probability.

Table 3c. Combined analysis of variance for capsule, and seed yield in jute mallow accessions during 2017 and 2018.

Source of variation	df	Number of seeds/capsule	Number of locules/capsule	Seed mass/ capsule (g)	100-seed weight (g)	Number of capsules/plant	Capsule mass (g)	Capsule length (cm)
Genotype	41	6703.70**	1393.90**	0.04**	0.01***	77412.44***	10599.71**	785.5**
Replicate (year)	3	2480.92**	5.30	0.007	0.006**	1078.63	308.51	8.20**
Genotype × year	83	193.51	6.40	0.001	0.003	425.56	463.63	84.86**
Error	165	177.35	12.29	0.004	0.25	4239.87	452.11	84.86

*, **0.05 and 0.01% levels of probability.

The top five accessions for leaf length, leaf width and number of leaves/plant are shown in Figures 1, 2, and 3. Accessions 25, 19, and 28 were pollen sources for leaf length, accessions 20, 27, and 1 for leaf width, accessions 31, 22, and 23 for the number of leaves/plant, and accessions 8, 11, and 7 for branch length. Considering seed metric traits, accessions 4, 18, and 27 outperformed the others for the number of seeds/capsule (Figure 4). A diallel crossing pattern among these accessions may recombine the genes for these traits and produce segregating populations for the high number of leaves/plant and seed mass for selection and variety development. The five accessions with the highest number of branches/plant at culling were accessions 11, 8, 20, 12, and 23, which had 71, 62, 54, 58 and 38 leaves/plant, respectively (Figure 5). The leaf chlorophyll content peaked (54.01) in accession 40, followed by accession 39 (52.75), accession 42 (53.58), accession 27 (53.53) and accession 25 (52.37).

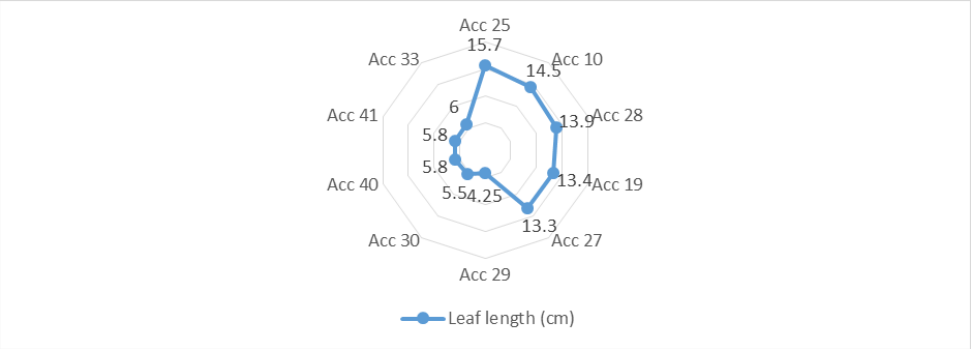

Figure 1. Leaf length (cm) at flowering among top and bottom five accessions of jute mallow during 2017 and 2018.

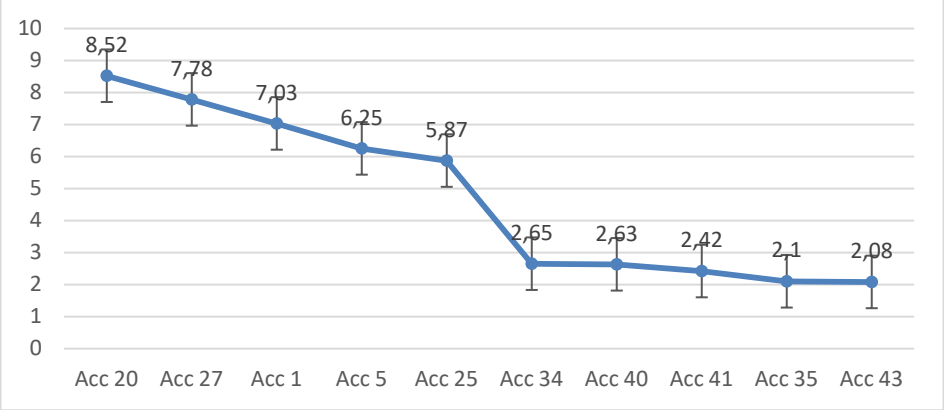

Figure 2. Leaf width (cm) among the top and bottom five accessions of jute mallow at flowering during 2017 and 2018.

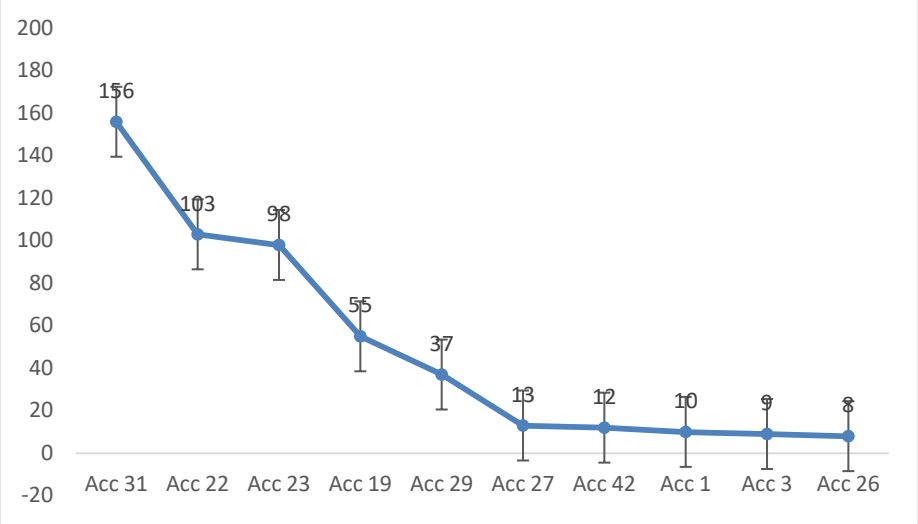

Figure 3. Number of leaves/plant among the top and bottom five accessions at flowering during 2017 and 2018.

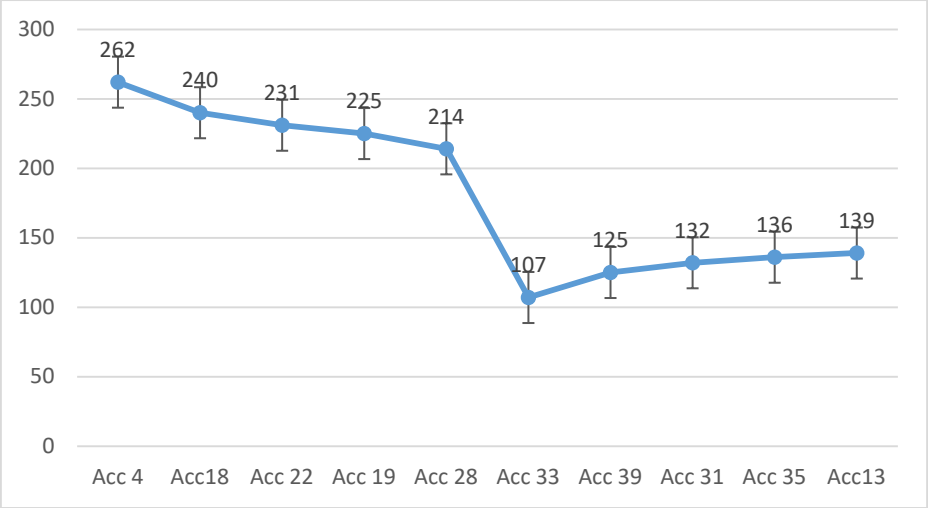

Figure 4. Number of seeds/capsule among top and bottom five accessions of jute mallow during 2017 and 2018.

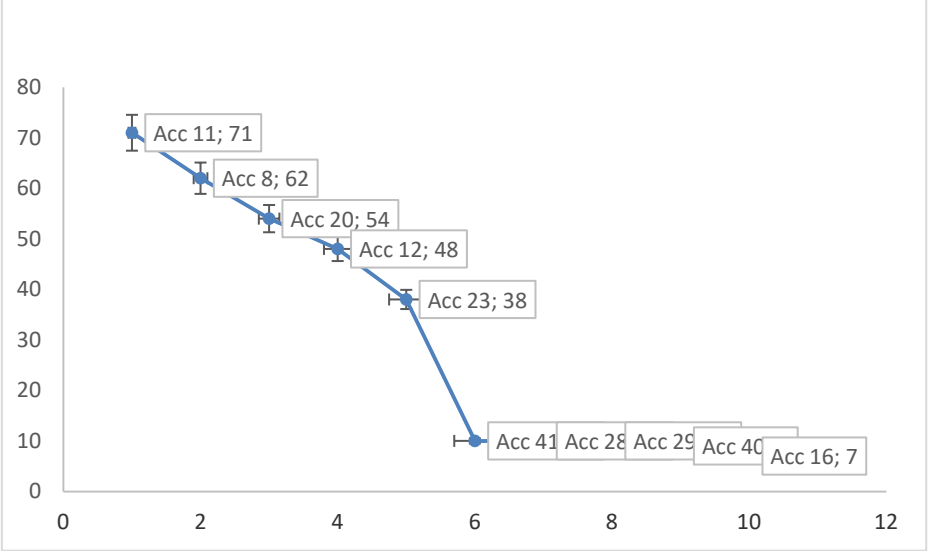

Figure 5. Number of branches/plant at culling among the top and bottom five accessions of jute mallow during 2017 and 2018.

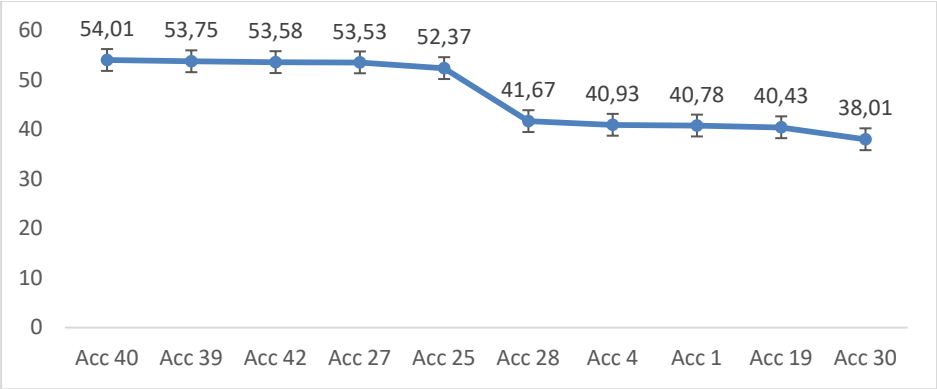

Figure 6. Leaf chlorophyll content among the top and bottom five accessions of jute mallow during 2017 and 2018.

Association between traits

Correlation between traits is important in crop improvement to enhance selection efficiency. Several authors have used the correlation coefficient, which measures the degree of association between traits for selection. The high degree of correlation between traits may be associated with the indirect effect of the third factor (Cruz, 2016). The number of leaves/plant was significantly correlated with leaf length (r=0.26*), leaf width (r= 0.12*), number of branches (r= 0.25*), plant height (r= 0.12*), and branch length (r= 0.25*) (Table 4). A positive association between seed mass and leaf length, leaf width, plant height at flowering and plant height at maturity suggests an efficient translocation of photo-assimilates produced in the leaves to the sink (number of seeds/capsule, number of seeds/capsule, 100-seed mass/capsule). A negative correlation coefficient between the number of leaves per plant and capsule length (r= -0.15*) and the number of capsules/plant (r= -0.13*) is similar to the previous report of Ngomuo et al. (2017) on *C. olitorius.* This suggests that fewer leaves were produced at the reproductive stage than at the vegetative stage. In contrast, Medisa et al. (2013) indicated a positive association between the number of leaves/plant and capsule mass. The leaves of vegetable crops are harvested early and consumed fresh. At post-anthesis stage, the photo-assimilates are channelled to the reproductive structures and sinks (seeds), producing only a few leaves/plant.

The seed mass was negatively correlated with leaf chlorophyll (r=-0.22*) and capsule length (r=-0.59**). An inverse relationship between the number of capsules/plant and seed mass showed effects on seed production. This suggests that some loculi within the capsules were seedless. In another study, Madakadze et al. (2007) indicated high seed mass in jute mallow. This was dependent on cultivar

and cultivation practices. On the other hand, the seed mass showed a positive correlation coefficient with leaf length (r=0.32*), leaf width (r=0.38*), plant height at flowering (r=0.13*), plant height at maturity (r=0.32*), number of seeds/capsule (r=0.37*), number of seeds/capsule (r=0.72**) and 100-seed mass/capsule (r=0.19*). The number of capsules/plant had a positive and significant correlation coefficient with leaf width (r=0.12*). The number of leaves/plant and leaf size (leaf length and width) are market traits for leafy vegetables. A significantly negative correlation coefficient between seed mass and capsule length suggests that capsule length is not an index of seed mass improvement. Moreover, the seed mass is the sink, while the capsule is an intermediate sink for photosynthates produced in the leaves. Accessions of jute mallow with a long capsule length may have few seeds per capsule. In another study, Panchbhaiya et al. (2017) found a negative correlation coefficient between seed yield and capsule length in common bean.

Contribution of agronomic traits to the number of leaves/plant and seed yield

The values for the indirect effect of the traits on the number of leaves/plant were generally low in magnitude compared to the direct effect (Table 5). The leaf length had the largest direct effect on the number of leaves/plant with its largest indirect effect through the reduction of seed mass. The leaf length showed a significantly positive correlation coefficient with seed mass (Table 4). A high direct effect for leaf length is consistent with the previous report of Tejaswini et al. (2017). Therefore, leaf length is a reliable index to improve the number of leaves/plant. The number of leaves/plant and seed yield are complex traits that are highly dependent on several other traits. The direct contribution of leaf width to the number of leaves was masked by other traits such as leaf chlorophyll, petal width, sepal length, flower size, branch length, and the number of capsules/plant. This justifies the competition for photo-assimilates between vegetative and reproductive traits. The leaf width and plant height at flowering indirectly enhanced the leaves produced on each plant. This may be associated with a positive and significant association between the number of leaves/plant, leaf length, and leaf width. In the crop improvement program, selection in favor of leaf length and leaf width will complement the number of leaves/plant. Considering traits with a negligible, low, and moderate negative direct effect on the number of leaves, selection of these traits may be ineffective in improving the number of leaves. The complementarity between leaf length and width for high leaf number is similar to the previous report of Nwangburuka and Denton (2012). Capsule length and seed mass masked the phenotypic expression of leaf number. A negative correlation coefficient between pistil height and the number of leaves/plant (r=-0.13*) suggests the limitation of selection of traits based on inter-trait association alone. The seed mass, 100-seed mass, and seed mass/capsule showed a high indirect contribution to the number of

leaves/plant. The sepal length had the largest negative direct effect on the number of leaves/plant at flowering and the largest negative indirect effect through a reduction in petal length. The residual factor (R^2=0.42) indicates that 68% of the total variation in the number of leaves/plant was explained by agronomic traits. A large contribution of branch length to the number of leaves/plant at flowering was masked by petal length and width, leaf width, plant height at flowering, and 100-seed mass. This demonstrates the importance of leaf length, pistil height, and leaf width as indices for improving the number of leaves/plant.

Table 4. Correlation coefficients between vegetative and seed yield traits.

Leaf chlorophyll	Leaf chlorophyll	Leaf length	Leaf width	Plant height at flowering	Plant height at flowering	Capsule mass	Capsule length	Days to 50% flowering	Number of locules	Number of seeds/ capsule	Seed mass
Leaf length	0.04										
Leaf width	-0.13*	0.48**									
Number of leaves/ plant	-0.007	0.26*	0.12*								
Plant height at flowering	0.008	0.30**	0.26*	0.07							
Capsule mass	-0.13*	-0.002	0.06	0.05	-0.11						
Capsule length	0.35**	-0.30**	-0.59**	-0.15*	-0.25*	-0.14*					
Days to 50% flowering	-0.38**	0.28*	0.50**	0.05	0.13*	0.09	-0.61**				
Number of locules/ capsule	-0.23**	0.22*	0.31**	-0.10	-0.002	0.07	-0.41**	0.59**			
Number of seeds/capsule	-0.32**	0.30**	0.55**	0.05	0.19*	0.19*	-0.78**	0.78**	0.58**		
Seed mass	-0.20*	0.32**	0.38**	-0.04	0.13*	-0.06	-0.31**	0.60**	0.37**	0.48**	
100-seed mass	-0.13*	0.2	0.27*	-0.02	0.07	0.01	-0.42**	0.44**	0.27*	0.4	0.19*
Petal length	0.01	0.26*	0.19*	-0.18*	0.29*	-0.11*	-0.32**	0.13*	0.12*	0.28**	0.19*
Petal width	0.09	-0.14	-0.06	-0.20*	-0.04	-0.24*	0.07	-0.16*	-0.03	-0.1	-0.02
Sepal length	0.18*	0.04	-0.008	-0.47**	0.06	-0.07	0.14*	-0.16*	0.009	-0.14*	0.006
Sepal width	0.05	0.0002	-0.05	-0.09	-0.01	-0.07	0.003	0.02	0.03	0.01	0.08
Stamen height	0.006	0.28*	0.33**	-0.11	0.17*	-0.05	-0.47**	0.28*	0.33**	0.46**	0.22*
Pistil height	-0.04	0.24	0.36**	-0.12*	0.2	0.02	-0.54**	0.34**	0.30**	0.52**	0.23*
Flower size	-0.09	-0.08	-0.01	-0.24*	-0.09	-0.06	-0.21*	0.08	0.14*	0.16*	0.06
Plant height at maturity	-0.09	0.02	0.03	0.12*	0.32**	-0.04	-0.11*	0.003	0.002	0.07	0.01
Number of branches/ plant	-0.09	0.005	0.26*	0.08	0.29	-0.06	-0.38**	0.39**	0.21*	0.38**	0.08
Branch length	-0.09	0.007	-0.11	0.25*	-0.15*	0.03	-0.08	0.013*	-0.13*	0.03	0.08
Number of capsules/ plant	-0.09	0.05	0.12*	-0.13*	-0.002	-0.13*	-0.19*	0.15	0.09	0.25*	0.007

Continuation Table 4. Correlation coefficients between vegetative and seed yield traits.

Leaf chlorophyll	100-seed mass	Petal length	Petal width	Sepal length	Stamen height	Pistil height	Flower size	Plant height at maturity	Number of branches/ plant	Branch length	Number of capsules/ plant
Leaf length											
Leaf width											
Number of leaves/ plant											
Plant height at flowering											
Capsule mass											
Capsule length											
Days to 50% flowering											
Number of locules/ capsule											
Number of seeds/capsule											
Seed mass											
100-seed mass											
Petal length	0.11										
Petal width	-0.20*	0.53**									
Sepal length	-0.08	0.46**	0.35**								
Sepal width	0.02	0.17*	0.12*	0.07							
Stamen height	0.15*	0.65**	0.40**	0.34**	0.06						
Pistil height	0.26*	0.75**	0.44**	0.38**	0.07	0.79**					
Flower size	0.06	0.62**	0.61**	0.34**	0.1	0.56**	0.70**				
Plant height at maturity	0.07	0.004	-0.01	-0.18*	0.03	-0.02	0.01	-0.06			
Number of branches/ plant	0.17*	-0.007	-0.13*	-0.14*	-0.08	0.08	0.09	-0.13*	0.17		
Branch length	-0.12*	-0.24*	-0.17*	-0.21*	-0.10	-0.01	-0.09	-0.02	0.05	0.06	
Number of capsules/ plant	0.12*	0.15*	-0.003	0.16	0.001	0.12*	0.26*	0.25	-0.02	0.05	0.06

Table 5. Direct and indirect effects of some characters on the number of leaves/plant.

Effect	Leaf length	Leaf width	Leaf chlorophyll	Plant height at flowering	Capsule length	Capsule width	Seed mass	Seed mass/capsule	100-seed mass
Direct effect	0.33	0.003	0.04	-0.032	-0.183	0.099	-0.217	-0.087	-0.122
Leaf length		0.001	0.002	-0.010	0.055	0.028	-0.056	-0.028	-0.025
Leaf width	0.16		-0.006	-0.008	0.110	0.050	-0.102	-0.034	-0.033
Leaf chlorophyll	0.01	0.000		0.000	-0.064	-0.038	0.048	0.018	0.016
Plant height at flowering	0.10	0.001	0.000		0.047	0.014	-0.033	-0.012	-0.010
Capsule length	-0.09	-0.002	0.015	0.008		-0.061	0.129	0.027	0.052
Capsule width	0.09	0.001	-0.017	-0.004	0.113		-0.145	-0.053	-0.054
Seed mass	0.09	0.001	-0.010	-0.005	0.109	0.066		-0.037	-0.038
Seed mass/capsule	0.11	0.001	-0.009	-0.004	0.057	0.060	-0.092		-0.024
100-seed mass	0.06	0.001	-0.006	-0.003	0.078	0.044	-0.067	-0.017	
Petal length	0.08	0.001	0.001	-0.010	0.060	0.013	-0.052	-0.017	-0.014
Petal width	-0.04	0.000	0.004	0.002	-0.014	-0.016	0.001	0.002	0.024
Sepal length	0.01	0.000	0.008	-0.002	-0.027	-0.016	-0.008	-0.001	0.010
Pistil height	0.08	0.001	-0.002	-0.007	0.100	0.034	-0.094	-0.021	-0.032
Flower size	-0.02	0.000	-0.004	0.003	0.039	0.008	-0.033	-0.006	-0.008
Number of branches/plant	0.002	0.001	-0.007	-0.010	0.069	0.039	-0.104	-0.007	-0.021
Branch length	0.002	0.000	-0.002	0.005	0.016	0.001	-0.004	0.007	0.015
Number of capsules/plant	0.02	0.000	-0.001	0.000	0.035	0.015	-0.055	-0.001	-0.016

Continuation Table 5. Direct and indirect effects of some characters on the number of leaves/plant.

Effect	Petal length	Petal width	Sepal length	Pistil height	Flower size	Number of branches/plant	Branch length	Number of capsules/plant
Direct effect	-0.046	0.056	-0.420	0.245	-0.156	0.065	0.133	-0.063
Leaf length	-0.012	-0.008	-0.017	0.060	0.013	0.000	0.001	-0.003
Leaf width	-0.009	-0.004	0.004	0.089	0.003	0.017	-0.015	-0.008
Leaf chlorophyll	-0.001	0.005	-0.076	-0.011	0.015	-0.011	-0.005	0.001
Plant height at flowering	-0.013	-0.003	-0.029	0.051	0.015	0.019	-0.021	0.000
Capsule length	0.015	0.004	-0.063	-0.134	0.033	-0.025	-0.011	0.012
Capsule width	-0.006	-0.009	0.070	0.084	-0.013	0.026	0.002	-0.010
Seed mass	-0.011	0.000	-0.016	0.106	-0.024	0.031	0.003	-0.016
Seed mass/capsule	-0.009	-0.002	-0.003	0.058	-0.010	0.005	-0.011	0.000
100-seed mass	-0.005	-0.011	0.034	0.064	-0.010	0.011	-0.016	-0.008
Petal length		0.030	-0.195	0.186	-0.098	0.000	-0.033	-0.010
Petal width	-0.025		-0.148	0.110	-0.096	-0.009	-0.023	0.000
Sepal length	-0.021	0.020		0.095	-0.055	-0.009	-0.028	-0.010
Pistil height	-0.035	0.025	-0.163		-0.110	0.006	-0.013	-0.017
Flower size	-0.029	0.035	-0.147	0.173		-0.008	-0.003	-0.016
Number of branches/plant	0.000	-0.008	0.059	0.023	0.020		0.009	-0.004
Branch length	0.011	-0.010	0.089	-0.024	0.003	0.004		-0.004
Number of capsules/plant	-0.007	0.000	-0.068	0.065	-0.039	0.004	0.009	

The number of branches/plant, seed mass/capsule, the number of seeds/capsule, and capsule mass showed a large positive direct effect on seed yield (Table 6). This was reduced by a high and negative direct effect of the number of leaves/plant, capsule length, and flower size. This implies that the number of branches/plant is a reliable index for seed yield improvement (Table 6). This is related to a positive association between branch length and seed mass.

Table 6. Path coefficient analysis depicting the direct and indirect effects of some traits on seed mass.

Effect	Number of branches/ plant	Seed mass/ capsule	Number of seeds/ capsule	Capsule mass	Number of leaves/plant	Plant height at flowering	Capsule length	100-seed mass	Petal length
Direct effect	0.26	0.22	0.19	0.17	-0.12	-0.05	-0.10	-0.005	-0.035
Number of branches/plant		0.086	0.042	0.066	-0.010	-0.013	0.039	-0.001	0.000
Seed mass/capsule	0.101		0.112	0.130	-0.007	-0.009	0.080	-0.002	-0.010
Number of seeds/capsule	0.058	0.131		0.098	0.012	0.000	0.042	-0.001	-0.004
Capsule mass	0.105	0.175	0.114		-0.006	-0.006	0.063	-0.002	-0.005
Number of leaves/plant	0.022	0.013	-0.020	0.009		-0.003	0.016	0.000	0.006
Plant height at flowering	0.078	0.044	0.0001	0.023	-0.009		0.026	0.000	-0.010
Capsule length	-0.100	-0.176	-0.080	-0.103	0.019	0.011		0.002	0.011
100-seed mass	0.046	0.090	0.053	0.074	0.003	-0.004	0.043		-0.004
Petal length	-0.002	0.064	0.024	0.022	0.022	-0.013	0.033	-0.001	
Petal width	-0.036	-0.022	-0.007	-0.027	0.024	0.002	-0.008	0.001	-0.019
Sepal length	-0.037	-0.032	0.002	-0.028	0.057	-0.003	-0.015	0.000	-0.016
Sepal width	-0.021	0.004	0.006	0.005	0.012	0.001	0.000	0.000	-0.006
Stamen length	0.022	0.103	0.064	0.047	0.014	-0.008	0.049	-0.001	-0.023
Pistil length	0.025	0.117	0.058	0.057	0.015	-0.009	0.056	-0.001	-0.026
Flower size	-0.034	0.038	0.029	0.014	0.030	0.004	0.022	0.000	-0.022
Plant height at maturity	0.046	0.016	0.0009	0.001	-0.014	-0.015	0.011	0.000	0.000
Branch length	0.017	0.007	-0.025	0.002	-0.031	0.007	0.009	0.001	0.009
Number of capsules/plant	0.015	0.058	0.019	0.026	0.017	0.000	0.020	-0.001	-0.006

Therefore, a phenotypic improvement in branch length will complement seed mass. The plant height at flowering and maturity enhanced branch length, and these traits were positively associated. Accessions with a high number of branches/plant showed a moderately high seed mass/plant. The direct and positive contribution of the number of seeds/capsule to seed mass was masked by the negative indirect effect of capsule length, petal width and branch length. Plant height both at flowering and maturity showed little influence on the number of seeds/capsule. A phenotypic selection directed toward the number of branches/plant will compensate for more flowers per plant, and consequently, high seed mass/plant. However, the

direct effect of the number of branches/plant had the largest negative indirect effect through capsule length, sepal length, petal width and flower size. The number of branches/plant had a significantly negative correlation coefficient (r=0.38**) with capsule mass. The direct path from the number of branches/plant to seed mass was masked by reproductive structures (capsule length, flower size, sepal length, and sepal width). This may be related to the demand for photo-assimilates by these reproductive organs. The direct path of seed mass was reduced by the negative indirect effect of the number of leaves/plant at flowering.

Continuation Table 6. Path coefficient analysis depicting the direct and indirect effects of some traits on seed mass.

Effect	Petal width	Sepal length	Sepal width	Stamen length	Pistil length	Flower size	Plant height at maturity	Branch length	Number of capsules/plant
Direct effect	0.119	0.063	-0.007	0.110	-0.034	-0.078	0.024	0.053	0.110
Number of branches/plant	-0.016	-0.009	0.001	0.009	-0.003	0.010	0.004	0.003	0.006
Seed mass/capsule	-0.012	-0.009	0.000	0.051	-0.018	-0.013	0.002	0.002	0.028
Number of seeds/capsule	-0.004	0.001	0.000	0.037	-0.010	-0.012	0.000	-0.007	0.011
Capsule mass	-0.019	-0.011	0.000	0.031	-0.012	-0.007	0.000	0.001	0.017
Number of leaves/plant	-0.024	-0.030	0.001	-0.013	0.004	0.019	0.003	0.014	-0.015
Plant height at flowering	-0.006	0.004	0.000	0.019	-0.007	0.007	0.008	-0.008	0.000
Capsule length	0.009	0.009	0.000	-0.053	0.019	0.017	-0.003	-0.004	-0.021
100-seed mass	-0.024	-0.005	0.000	0.017	-0.009	-0.005	0.002	-0.006	0.014
Petal length	0.064	0.029	-0.001	0.072	-0.026	-0.049	0.000	-0.013	0.017
Petal width		0.022	-0.001	0.045	-0.015	-0.048	0.000	-0.009	0.000
Sepal length	0.042		-0.001	0.038	-0.013	-0.027	-0.005	-0.011	0.018
Sepal width	0.015	0.005		0.007	-0.003	-0.008	0.001	-0.005	0.000
Stamen length	0.048	0.022	0.000		-0.027	-0.044	-0.001	-0.001	0.014
Pistil length	0.053	0.025	-0.001	0.087		-0.055	0.000	-0.005	0.029
Flower size	0.073	0.022	-0.001	0.062	-0.024		-0.001	-0.001	0.028
Plant height at maturity	-0.002	-0.012	0.000	-0.003	0.000	0.005		0.003	-0.002
Branch length	-0.021	-0.013	0.001	-0.002	0.003	0.002	0.001		0.007
Number of capsules/plant	0.000	0.010	0.000	0.014	-0.009	-0.020	0.000	0.004	

The inverse relationship between capsule length and seed mass is consistent with the masking action of capsule length on the direct path to seed yield. The sepal length masked the phenotypic expression of the number of leaves/plant, and this was further enhanced by a negative indirect effect on petal length. This is consistent with the negative association between these traits. The number of leaves/plant showed a moderate and negative direct effect on seed mass. This may be ascribed to low to moderate leaves retained by the plant at seed maturity. The direct effect of the number of branches/plant on seed mass was evident in the mean

performance for seed mass and branch length, with accessions 11 and 8 performing best for both traits. Similarly, accessions 22 and 19 performed best for leaf length and number of leaves/plant. This indicates complementarity between both traits for the number of leaves. The residual (42 %) indicates that 68 % of the total variation in seed mass (dependent variable) was explained by other traits.

Conclusion

The study showed significant differences among the accessions of jute mallow in terms of agronomic, capsule, and seed yield traits. Promising accessions for leaf number/plant (accession 31), leaf length (accession 25), leaf width (accession 20), number of seeds/capsule (accession 4), and leaf chlorophyll (accession 40) are potential pollen parents for hybridization to develop a segregating population providing high leaf number, leaf chlorophyll and seed yield for selection. Phenotypic improvement in leaf length will enhance the number of leaves per plant. Direct selection of the number of branches/plant, the number of seeds/capsule, and the seed mass will improve seed yield. Accession 31 with five times the mean for the number of leaves/plant, and accession 22 with three times the mean for the number of leaves/plant are recommended for further genetic improvement.

Acknowledgements

We acknowledge the National Center for Genetic Resources and Biotechnology, Ibadan Nigeria for providing seeds and Dr. Ukoabasi Ekanem for data analysis.

References

Anonymous (2008). Asian Vegetable Research and Development Center, (AVRDC-The World Vegetable Center, Shanhua, Taiwan). Genetic Resources and Seed Unit (GRSU). Characterization records sheets.

Adebo, H., Ahoton, L., Quenum, F., & Ezin, V. (2015) Agro morphological characterization of *Corchorus olitorius* Cultivars of Benin. *Annual Research and Review in Biology*, *7* (4), 229-240.

Benor, S., Demissew, S., & Hammer, K. (2012). Genetic diversity and relationships in *Corchorus olitorius* (Malvaceae s.l.) inferred from molecular and morphological data. *Genetic Resources and Crop Evolution*, *59*, 1125-1146.

Cruz, C.D. (2016). Genes Software - extended and integrated with the R, Matlab and Selegen. *Acta Scientiarum. Agronomy*, *38* (4), 547-552.

Dewey, D.R., & Lu, K.H. (1959). A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agronomy Journal*, *51*, 515-518.

Fasinmirin, J.T., & Olufayo, A.A. (2009). Yield and water use efficiency of jute mallow *Corchorus olitorius* under varying soil water management strategies. *Journal of Medicinal Plants Research*, *3*, 186-191.

Grubben, G.J.H., & Denton, O.A. (2004). Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands.

Ghosh, S., Meena, K., & Sinha, M.K. (2017). Genetic diversity in *Corchorus olitorius* genotypes using jute SSRs. *National Academic of Science, 87* (3), 917-926.

Lewu, F.B., & Mavengahama, S. (2010). Wild vegetables in northern Kwazulu Natal, South Africa: Current status of production and research needs. *Scientific Research and Essays*, *5*, 3044-3048.

Madakadze, R.M., Kodzanayi, T., & Mugumwa, R. (2007). Effect of plant spacing and harvesting frequency on *Corchorus olitorious* leaf and Seed yields. In Ahmed, K.Z. (Eds.), *Proceedings of* the *African crop science society, 8*, (pp. 279-282). El-Minia.

Medisa, M.E., Mathowa, T., Mpofu, C., Ndapo, S., & Machacha, S. (2013). Effect of chicken manure and commercial fertilizer on performance of jute mallow (*Corchorus olitorius*). *Agriculture and Biology Journal of North America, 4*, 617-622.

Ngomuo, M.S., Stoilova, T., Feyissa, T., Kassim, N., & Ndakidemi, P.A. (2017). Leaf and seed yield of jute mallow (*Corchorus olitorius* L.) accessions under field conditions for two consecutive growing seasons. *Journal of Horticultural Science and Biotechnology*. DOI: 10.1080/14620316.2017.1304168

Panchbhaiya, A., Singh, D.K., & Jain, S.K. (2017). Inter-characters association studies for morphological, yield and yield attributes in the germplasm of French bean (*Phaseolus vulgaris* L.) in Tarai region of Uttarakhand, India. *Legume Research-An International Journal, 40* (1), 196-199.

Nwangburuka, C.C., & Denton, A. (2012). Heritability, trait association and genetic advance in six agronomic and yield related traits in leaf *Corchorus olitorius. International Journal of Agricultural Research, 7*, 367-375.

Tejaswini, N., Ravinder, K., Reddy, I., Saidaiah, P., & Ramesh, T. (2017). Correlation and Path Coefficient Analysis in Vegetable Amaranth (*Amaranthus tricolor* L.) Genotypes. *International Journal of Current Microbiology and Applied Sciences, 6* (6), 2977-2996.

Opabode, J.T., & Adebooye, O.C. (2005). Application of biotechnology for the improvement of Nigerian indigenous leaf vegetables. *African Journal of Biotechnology*, *4*, 138-142.

Whitlock B. A.W., Karol, K.G., & Alverson, W. S. (2003). Chloroplast DNA sequences confirm the placement of the enigmatic *Oceano papaver* within Corchorus (formerly Tiliaceae). *International Journal of Plant Science*, 164, 35-41.

Wright, S. (1921). The methods of the path coefficients. *The Annals mathematics and Statistics*, *5*, 161-215.

Received: October 12, 2023
Accepted: April 8, 2024

FENOTIPSKA VARIJACIJA I SIMULTANA SELEKCIJA BROJA LISTOVA/BILJCI I MASE SEMENA KOD JUTENOG SLEZA (*CORCHORUS OLITORIUS*)

Adekoya M. Adejoke[1*], Adeniji O. Taiwo[2], Ekanem U. Okon[3], Badmus A. Adeshile[2], Peter J. Melangu[4] i Olosunde O. Busayo[2]

[1]Department of Plant Science and Biotechnology, Federal University, Oye Ekiti, Nigeria
[2]Department of Crop Science and Horticulture,
Federal University Oye Ekiti, Ekiti State, Nigeria
[3]International Institute of Tropical Agriculture, Ibadan, Nigeria
[4]Adamawa State University, Mubi, Nigeria

R e z i m e

Corchorus olitorius je lisnato povrće koje se uzgaja zbog sluzi u listovima. Zelenilo listova, broj listova, dužina lista i širina lista su popularne karakteristike na tržištu ovog povrća. Malo se zna o direktnom i indirektnom doprinosu karakteristika kao što su broj listova i prinos semena. Procenjena su četrdeset i dva genotipa u randomizovanom kompletnom blok dizajnu sa četiri ponavljanja tokom berbe 2017. i 2018. godine. Rezultati su pokazali da su genotipovi 25, 19 i 28 imali najveću dužinu lista, genotipovi 31, 22 i 23 broj listova po biljci, genotipovi 4, 18 i 27 broj semena po kapsuli i genotipovi 8, 11 i 7 masu semena. Masa semena je bila u pozitivnoj korelaciji sa dužinom lista, širinom lista i visinom biljke u zrelosti, brojem semena po kapsuli i masom 100 semena. Na broj listova po biljci uticala je dužina lista, širina lista i dužina grane. Pat analiza za masu semena pokazala je da broj grana po biljci, masa semena po kapsuli, broj semena po kapsuli i masa kapsule značajno doprinose prinosu semena. Indirektan doprinos karakteristika broju listova po biljci bio je mali u poređenju s direktnim uticajem. Dužina lista je imala najveći direktni uticaj na broj listova po biljci dok je njen najveći indirektni uticaj bio smanjenje mase semena. Direktni doprinos dužine lista broju listova po biljci bio je maskiran fenotipskom ekspresijom širine latice. Broj grana po biljci je pouzdan indeks za poboljšanje prinosa semena. Hibridizacija genotipova s najboljim učinkom za broj listova, sadržaj hlorofila u listu i prinos semena proizvešće nove sorte putem selekcije.

Ključne reči: juteni slez, fenotipska varijacija, korelacija karakteristika, selekcioni indeks, poboljšanje prinosa.

Primljeno: 12. oktobra 2023.
Odobreno: 8. aprila 2024.

[*]Autor za kontakt: e-mail: munawarm11@gmail.com

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2402155O
Vol. 69, No. 2, 2024 UDC: 633.82:582.568.4
Pages 155-168 Original scientific paper

RESPONSE OF MANGO GINGER (*CURCUMA AMADA*) TO PLANT POPULATION AND DIFFERENT WEED CONTROL METHODS IN THE FOREST-SAVANNA TRANSITION ZONE OF SOUTH-WESTERN NIGERIA

Samuel O. Osunleti[1,2*] and Segun T.O. Lagoke[2]

[1]Department of Agricultural Technology, Edo State College of Agriculture and Natural Resources, Iguoriakhi, Edo State, Nigeria
[2]Department of Plant Physiology and Crop Production, Federal University of Agriculture, Abeokuta, Alabata, Abeokuta, Ogun State, Nigeria

Abstract: Field trials were conducted in the early cropping seasons of 2016 and 2017 at the Teaching and Research Farm of Federal University of Agriculture, Abeokuta (07° 20' N, 3° 23' E 159 m above sea level) in the forest-savanna transition zone of south-western Nigeria to evaluate the response of mango ginger to plant population and different weed control methods. Treatments were laid out in a randomized complete block design with a split-plot arrangement and replicated three times. The main plot consisted of two plant populations: 444,444 plants/ha and 250,000 plants/ha, while the sub-plots consisted of ten weed control methods. The collected data on growth and yield of mango ginger plant, and weed biomass were subjected to analysis of variance (ANOVA) and the means of the treatments were separated using the least significant difference (LSD at $p \leq 0.05$). Planting mango ginger at 444,444 plants/ha resulted in a 43.1% increase in rhizome yield compared to 250,000 plants/ha. Different weed control methods gave significantly higher crop vigor score, yield and yield components than the weedy check. Relative to the highest value in both years, uncontrolled weed infestation resulted in a 91.4% reduction in rhizome yield. There was a 60.7% increase in mango ginger rhizome yield when post-emergence weed control followed pre-emergence weed control. Our study has revealed that mango ginger, as a perennial crop with initially slow growth, requires a weed-free period beyond the first 12 weeks after planting (WAP) for acceptable weed control and optimum rhizome yield. Hence, a pre-emergence application of oxyfluorfen at a dosage of 0.24 kg a.iha^{-1} and a post-emergence hoe weeding are recommended.

Key words: mango ginger, rhizomes, population, post-emergence, weed infestation.

*Corresponding author: e-mail: osunletis@gmail.com

Introduction

Mango ginger (*Curcuma amada* Roxb.) is a rhizomatous perennial plant belonging to the Zingiberaceae family with leafy tuft and of Asian origin (Chatterjee et al., 2012). The fresh rhizome of mango ginger looks like ginger (*Zingiber officinale*) but smells like mango (*Mangifera indica*) and is therefore known as mango ginger (Sasikumar, 2005). The plant is mainly cultivated in tropical areas, especially in India, for its rhizomes, which are used as a mild ginger spice and also have medicinal uses (Huxley, 1992; Chevallier, 1996). Mangiferin is a phytochemical found in mango ginger, and has been shown to have anti-inflammatory, anti-diabetic, anti-cancer, immunomodulatory, anti-HIV, and anti-oxidant properties (Wauthoz et al., 2007; Padmapriya et al., 2012). In addition, the plant is said to have antiobesity (Nissankara-Rao et al., 2021), antitumor (Ramachandran et al., 2017), and antibacterial (Divyashri et al., 2021) effects. The chemical composition and numerous biological activities of the essential oil produced by hydrodistillation have also been evaluated. The hydrodistilled essential oil from *Curcuma amada* has been reported to be a good source of pinene, ocimene, curcumene, and linalool (Padalia et al., 2013; Srivastava et al., 2001; Tamta et al., 2016). The essential oil has been shown to have antifungal properties against a range of fungi, including *Aspergillus species* (Singh et al., 2002).

Despite its widespread importance, the productivity of mango ginger remains low due to factors such as poor agronomic practices and uncontrolled weed infestation (Hailemichael and Tesfaye, 2008; MoARD, 2007; Osunleti et al., 2021b). Plant population has been identified as an important factor contributing significantly to the growth and yield of mango ginger (Osunleti et al., 2023). Bahadur et al. (2000) previously reported appreciable differences in growth and yield of turmeric when the spacing between plants was varied. Hailemichael and Tilahun (2004) recommended 222,000 plants per hectare at 15 cm x 30 cm spacing in a preliminary study conducted on the effect of plant population density of ginger on rhizome yield at Tapi, western Ethiopia. Osunleti et al. (2023) observed higher rhizome yield in mango ginger when the population increased beyond 222,000 plants.

Pests are important organisms that have a significant effect on the agriculture system, health systems, and economic status of the country (Farnsworth et al., 2017). Weeds have been described as the most common crop pests in the humid and subtropical countries (Nedunchezhiyan et al., 2013), competing with crops for water, soil nutrients, light and even harboring insect pests (Unamma et al., 1984; Osunleti et al., 2021a). Eshetu and Addisu (2015) observed a 100 percent yield loss when weed control was completely ignored. Uncontrolled weed infestation throughout the crop life cycle has earlier been reported to cause 85.1% to 92.2% rhizome yield loss in mango ginger (Osunleti et al., 2021a, b). In most cases, weed

management accounts for the major share of the total cost and time of production (KAU, 2006; Osunleti et al., 2022b). The mango ginger plant has been reported to belong to the same family as ginger (Zingiberaceae), and is closely related to turmeric (Chandarana et al., 2005). Several studies have been carried out on different weed control measures in turmeric (Sathiyavani and Prabhakaran, 2015; Suresh et al., 2014) and ginger (Baruah and Deka, 2020), but there is a dearth of information on weed control measures in mango ginger. Moreover, the initial slow growth of mango ginger makes it highly vulnerable to weed infestation with high yield losses (Osunleti et al., 2023). Therefore, different weed control strategies must be tested to ensure an initial weed-free period for mango ginger and minimize yield losses.

Material and Methods

Site description

Field experiments were conducted at the Federal University of Agriculture, Abeokuta, Nigeria, between June and December during the 2016 and 2017 cropping seasons to determine the response of mango ginger to plant population and different weed control methods. The details of the physico-chemical properties of the soil before the commencement of the trials are included in Table 1. The result of the soil analysis showed that the soil had a sandy loam texture in both years with a pH of 6.5 and 6.6 in 2016 and 2017, respectively (Table 1). The site received a total rainfall of 1146.3 and 839.7 mm during the 2016 and 2017 growing seasons, respectively (Table 2).

Table 1. Physico-chemical properties of soil at the experimental sites in 2016 and 2017.

Soil composition	2016	2017
pH	6.5	6.6
Particle size analysis		
Sand (g/kg)	799.5	736.6
Silt (g/kg)	160.5	220.5
Clay (g/kg)	40.0	42.9
Textural class	Sandy loam	Sandy loam
Chemical composition		
Organic carbon (%)	1.56	1.92
Available P (mg/kg)	2.46	4.05
Total N (%)	0.11	0.13
Exchangeable cations (centimol/kg)		
Ca	6.64	6.59
Na	0.22	0.16
Mg	1.25	0.18
K	0.31	0.45

Table 2. Monthly distribution of total rainfall and mean temperature of the experimental site in 2016 and 2017.

Month	2016		2017	
	Total rainfall (mm)	Mean temperature (°C)	Total rainfall (mm)	Mean temperature (°C)
January	32.0	28.1	15.9	28.9
February	0.0	30.3	0.0	30.2
March	150.3	29.5	34.3	30.0
April	68.2	29.2	112.8	29.1
May	226.2	29.0	146.0	27.8
June	150.5	26.7	111.0	26.7
July	65.2	26.3	156.0	25.7
August	63.6	25.7	90.0	25.5
September	229.0	26.9	52.0	25.2
October	155.4	28.0	90.2	27.6
November	5.9	22.5	45.6	28.6
December	0.0	28.1	15.9	28.9
Mean		27.5		27.9
Total	1146.3		839.7	

Source: Federal University of Agriculture, Abeokuta Meteorological Station.

Experimental design

Mango ginger rhizomes were sown in June of the 2016 and 2017 cropping seasons using a split-split plot design with three replicates. Two plant spacings (0.15 m x 0.15 m and 0.20 m x 0.20 m) were used in both years, giving a plant population of 444,444 and 250,000 plants/ha, respectively, assigned to the main plots. Ten weed control methods were assigned to the sub-plots: pre-emergence application of oxyfluorfen (24% EC) at 0.36 kg a.iha^{-1} + hoe weeding as a post-emergence treatment (TT1), pre-emergence application of oxyfluorfen at 0.36 kg a.iha^{-1} + (oxadiazon 25% EC at 0.5 kg a.iha^{-1} and diuron 50% EC at 0.4 kg a.iha^{-1}) as a post-emergence treatment (TT2), pre-emergence application of oxyfluorfen at 0.36 kg a.iha^{-1} alone (TT3), pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} + hoe weeding as a post-emergence treatment (TT4), pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} + (oxadiazon 25% EC at 0.5 kg a.iha^{-1} and diuron 50% EC at 0.4 kg a.iha^{-1}) as a post-emergence treatment (TT5), pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} alone (TT6), hoe weeding at 4, 8 and 12 WAP (TT7), hoe weeding at 4, 8, 12 and 16 WAP (TT8), hoe weeding at 4, 8, 12, 16 and 20 WAP (TT9) and a weedy check, where no weeding was applied (T10). The post-emergence application was carried out at 8 weeks after planting.

Cultural practices

The experimental site was plowed and harrowed at two-week intervals each cropping season to destroy the existing vegetation and produce level, smooth and weed-free fields. After the removal of weed debris, the land was divided into various replicates, plots and subplots. The mango ginger rhizomes were then planted into various plots (0.15 m x 0.15 m and 0.20 m x 0.20 m) according to the treatment plan. The pre-emergence herbicide oxyfluorfen (24% EC) was applied, while some plots were left without the pre-emergence herbicide application according to the treatment structure. Manual weeding with the West African hand hoe was carried out according to the treatment requirements.

Data collection

1. The crop vigor score was determined on a scale of 1 to 10, where 1 means complete crop death and 10 means a vigorously growing crop. The components of crop vigor score included: height of the crop, greenness of the leaves, and length and width of the leaves.
2. The rhizomes were harvested from the net plot, counted and weighed, and recorded as the number of rhizomes and yield, respectively (at 6 months after planting).
3. Weed biomass: Weed samples were collected in squares of 0.5 m × 0.5 m (2 in each plot) before any weeding. The samples taken from each plot were separated into broadleaves, grasses and sedges. The samples were oven-dried at 70°C until a constant dry weight was obtained and weighed.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Genstat 12th edition to determine the level of significance of the treatments. The treatment means were separated using the least significant difference (LSD at $p \leq 0.05$).

Results and Discussion

Growth parameters

During the entire observation period, the weed control methods had a significant effect on the crop vigor score (Table 3). At 6 WAP in both years, the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} + hoe weeding as a post-emergence treatment resulted in the highest crop vigor score, while the lowest crop vigor in 2017 was recorded in the weedy check. At 9 and 12 WAP in both

years, the lowest crop vigor score was recorded in the weedy check plots. At 9 WAP in both years and 12 WAP in 2017, the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} + hoe weeding as a post-emergence treatment resulted in significantly higher crop vigor than all other treatments except for the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} + oxadiazon and diuron as a post-emergence treatment. The lowest crop vigor score in the weedy check plots in this study was due to the intense weed competition with the crops. The weeds competed freely with the crops for light, space, soil nutrients and moisture, thereby limiting the resources available for the crops for proper growth. Mango ginger has been reported to be a slow-growing crop initially, and highly susceptible to weed infestation (Osunleti et al., 2023; Osunleti et al., 2021a). An initial weed-free period is a necessity for a good start and healthy growth of mango ginger. Our results showed that the application of pre-emergence herbicides or early hoe weeding ensured a weed-free period and increased the crop vigor. This agrees with the findings (Osunleti et al., 2023; Osunleti et al., 2021b), which described higher crop vigor in mango ginger as a result of early weed removal in mango ginger using a hoe and the application of pre-emergence herbicides.

Table 3. Effects of plant population and weed control methods on crop vigor score.

Treatments	Crop vigor score					
	6 WAP		9 WAP		12 WAP	
	2016	2017	2016	2017	2016	2017
Plant population (P)						
444,444	2.8	2.9	4.9	5.2	6.0	6.0
250,000	2.9	3.0	4.9	5.1	6.0	6.0
Lsd	0.932ns	0.502ns	0.072ns	0.399ns	0.124ns	0.124ns
Weed control methods (W)						
TT1	2.9	3.0	5.1	5.3	6.0	6.1
TT2	2.8	3.0	5.0	5.3	6.3	6.3
TT3	2.7	2.7	4.8	5.2	5.8	5.8
TT4	3.3	3.4	5.5	5.8	6.7	6.8
TT5	2.8	3.0	5.3	5.7	6.5	6.8
TT6	2.8	2.8	5.2	5.3	5.9	6.0
TT7	3.0	3.0	4.7	5.2	6.3	6.3
TT8	2.8	3.0	5.0	5.3	6.3	6.3
TT9	2.7	3.0	4.8	5.2	6.3	6.3
TT10	2.5	2.5	3.2	3.1	3.3	3.3
Lsd	0.268	0.294	0.377	0.302	0.328	0.297
Interaction						
P * W	ns	ns	ns	ns	ns	ns

Yield and yield components

Plant population had no significant effect on the yield and yield components of mango ginger (Table 4). However, planting mango ginger at 444,444 plants/ha resulted in a 43.1% increase in rhizome yield in both years compared to 250,000 plants/ha. The higher yield with 444,444 plants/ha could be attributed to the higher stands of the crop on the plots. Similar findings were observed by Bahadur et al. (2000), who reported a higher rhizome yield of turmeric at closer spacing and attributed this to a higher plant population per unit area.

The weed control methods had significant effects on the yield and yield components of mango ginger (Table 4). In both years, the lowest rhizome yield and count were recorded in the weedy check plot. The pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} and 0.36 kg a.iha^{-1} + hoe weeding as a post-emergence treatment, and plots hoe weeded five times at 4, 8, 12, 16 and 20 WAP produced similar rhizome yields, which were significantly higher than the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} and 0.36 kg a.iha^{-1} alone, and hoe weeding at 4, 8 and 12 WAP. Relative to the weedy check in both years, rhizome yield increased by 479% to 1067% by the various weed control methods, with the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} + hoe weeding as a post-emergence treatment having the highest value of 1067% (Figure 1). Hoe weeding at 4, 8, 12, 16 and 20 WAP and the pre-emergence application of oxyfluorfen at 0.36 kg a.iha^{-1} + hoe weeding as a post-emergence treatment also increased rhizome yield by 1063% and 1018% compared to the weedy check (Figure 1). The higher yield of mango ginger with the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} and 0.36 kg a.iha^{-1} plus hoe weeding as a post-emergence treatment could be attributed to both initial and subsequent weed-free periods provided by the treatment. The pre-emergence application of oxyfluorfen provided the initial weed-free period, while the subsequent weed-free period was achieved by the hoe weeding. The long weed-free period in the plots with the application of pre-emergence herbicides and hoe weeding gave the crop the opportunity to maximize its potential as it had the opportunity to adequately utilize the soil nutrients. Sivakumar et al. (2019) took similar views and reported more ginger rhizomes when the pre-emergence herbicide was followed up with hand weeding. Barla et al. (2015) also concluded that the application of metribuzin as a pre-emergence herbicide followed by a post-emergence treatment was the most productive among the different weed control methods experimented. In our study, a 60.7% higher yield of mango ginger rhizomes was observed when post-emergence weed control followed pre-emergence weed control, compared to pre-emergence herbicide application alone. This is a further indication that mango ginger requires more than the initial weed-free period for optimum yield in the crop. There was a 91.4% reduction in yield of mango ginger due to uncontrolled weed infestation

throughout the growing season. This confirms that mango ginger is a poor competitor. This result is similar to that of Krishnamurthy and Ayyaswamy (2000), who reported a 75% reduction in the yield of turmeric, while Osunleti et al. (2021a) reported an 85.1% reduction in yield due to seasonal competition with weeds.

Table 4. Effects of plant population and weed control methods on yield and yield components of mango ginger.

Treatments	Rhizome yield (kg/ha)		Number of rhizomes (x0000/ha)	
	2016	2017	2016	2017
Plant population (P)				
444,444	25769	30915	806	1007
250,000	18656	20944	663	731
Lsd	8854.3ns	15500.5ns	513.1ns	496.4ns
Weed control methods (W)				
TT1	30855	34119	988	1099
TT2	22554	25407	735	818
TT3	14961	23304	626	643
TT4	31956	35862	941	1162
TT5	27897	31268	923	1024
TT6	15063	21331	646	799
TT7	16045	17611	640	837
TT8	28169	31599	675	1015
TT9	31888	35722	1072	1189
TT10	2738	3074	97	107
Lsd	7632.2	9459.2	327.3	293.8
Interaction				
P * W	ns	ns	ns	ns

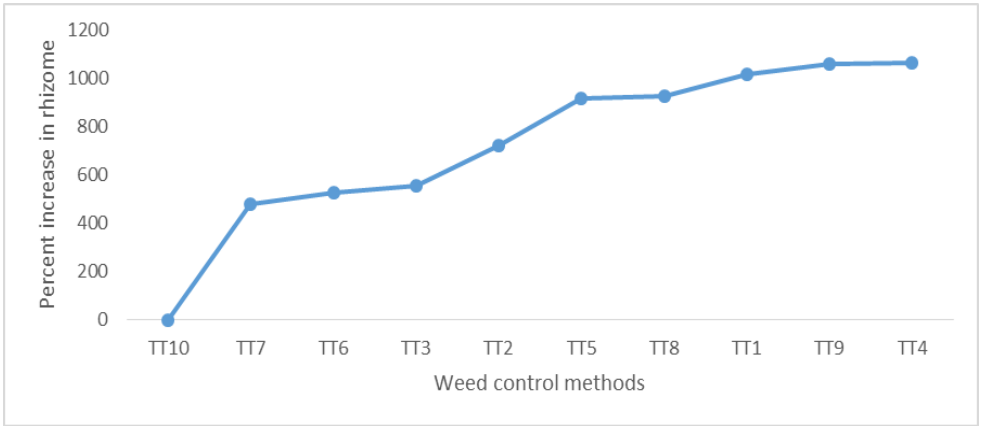

Figure 1. Effects of weed control methods on the increase in rhizome yield.

In both years, hoe weeding at 4, 8, 12, 16 and 20 WAP resulted in a significantly higher number of rhizomes than hoe weeding at 4, 8 and 12 WAP and the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} and 0.36 kg a.iha^{-1} alone (Table 4). The higher number of rhizomes in the plots hoe weeded at 4, 8, 12, 16, and 20 WAP was the result of continuous hoe weeding in the plots, leading to long-lasting weed control. This allows the opportunity for optimal utilization of the environmental resources required for good crop growth. The earlier study of Salawudeen (2017) also showed that the weed-free period beyond 12 WAP in mango ginger as a result of hoe weeding increased the yield of the crop.

Weed parameters

Weed control methods had a significant effect on weed biomass at 8 and 24 WAP (Tables 5 and 6). During the entire observation period at 8 and 24 WAP, the highest weed biomass was recorded in the weedy check plots. In 2017 at 8 WAP, the pre-emergence application of oxyfluorfen at both rates resulted in significantly lower biomass of broadleaves and grasses than in the hoe weeding plots. The pre-emergence application of the herbicide resulted in the complete control of sedges. The lower weed cover and biomass in the herbicide-treated plots could be attributed to the application of pre-emergence herbicides at planting, as the herbicide bombed the weed seeds and prevented their germination.

Table 5. Effects of plant population and weed control methods on weed biomass at 8 WAP.

Treatments	Weed biomass (kg/ha)					
	Broadleaves		Grasses		Sedges	
	2016	2017	2016	2017	2016	2017
Plant population (P)						
444,444	408	495	463	324	88	72
250,000	365	470	423	336	100	60
Lsd	291.9ns	359.7ns	397.0ns	195.9ns	74.3ns	48.8ns
Weed control methods (W)						
TT1	124	10	74	51	0	0
TT2	108	62	93	51	0	0
TT3	108	57	93	53	0	0
TT4	82	114	134	87	0	0
TT5	159	114	123	108	0	0
TT6	136	118	95	102	0	0
TT7	369	945	424	635	147	114
TT8	362	777	276	616	193	87
TT9	329	876	286	491	146	137
TT10	2090	1758	2834	1106	463	324
Lsd	211.4	236.7	232.1	169.4	58.8	63.7
Interaction						
P * W	ns	ns	ns	ns	ns	ns

This result corroborates the findings of Osunleti et al. (2022a), who reported a lower weed infestation when pre-emergence herbicides were applied compared to hoe weeding at 8 weeks after planting. In addition, Ramalingam et al. (2013) reported lower weed count and biomass with pre-emergence applications of oxyfluorfen. The integration of pre-emergence herbicides and hoe weeding as a post-emergence treatment resulted in the lowest weed cover at 12 WAP. Several researchers, including Osunleti et al. (2022b) and Ramalingam et al. (2013), had previously reported lower weed cover with pre-emergence herbicide application and supplementary hoe weeding.

Table 6. Effects of plant population and weed control methods on weed biomass at 24 WAP.

Treatments	Weed biomass (kg/ha)					
	Broadleaves		Grasses		Sedges	
	2016	2017	2016	2017	2016	2017
Plant population (P)						
444,444	719	733	609	811	274	343
250,000	752	646	542	845	291	378
Lsd	106.0ns	242.7ns	164.7ns	115.9ns	57.7ns	92.0ns
Weed control methods (W)						
TT1	96	110	92	107	43	55
TT2	120	137	115	134	52	66
TT3	686	751	606	790	364	431
TT4	107	122	103	119	43	40
TT5	108	124	104	121	43	55
TT6	707	813	673	814	326	445
TT7	658	714	600	737	328	420
TT8	235	348	292	264	146	187
TT9	103	143	120	115	50	74
TT10	4534	3632	3052	5078	1435	1836
Lsd	295.4	382.7	322.9	329.7	222.9	282.6
Interaction						
P * W	ns	ns	ns	ns	ns	ns

At 24 WAP in both years, the pre-emergence application of oxyfluorfen at both rates plus various post-emergence treatments resulted in significantly lower weights of broadleaves, grasses and sedges than the pre-emergence application of oxyfluorfen at both rates alone and hoe weeding at 4, 8 and 12 WAP (Table 6). Similarly, hoe weeding after 12 WAP resulted in significantly lower weed biomass than weeding at 4, 8 and 12 WAP and the pre-emergence application of oxyfluorfen alone at both rates. There was a 85% decrease in weed biomass at 24 WAP with the application of a pre-emergence herbicide plus a post-emergence treatment compared to the application of a pre–emergence herbicide alone. This indicates the importance of the post-emergence treatment, especially for a long-

standing crop such as mango ginger. Similarly, there was a 57% decrease in weed biomass at 24 WAP with hoe weeding at 4, 8, 12 and 16 WAP compared to weeding at 4, 8 and 12 WAP, while there was a further 25% decrease with weeding up to 20 weeks. The lengthened weed-free period in the plots with pre-emergence herbicide applications and post-emergence treatments, as well as plots weeded beyond 12 WAP are an important factor for the higher rhizome yields recorded in the plots. Sah et al. (2017) also reported effective and long-term weed control by applying oxyfluorfen in combination with hand weeding in ginger.

Conclusion

The results of this study showed that planting mango ginger at 444,444 plants/ha produced 43.1% more than at 250,000 plants/ha. Therefore, planting mango ginger at 444,444 plants/ha is recommended. The study also showed that different weed control methods had different effects on growth, yield of mango ginger and weed infestation. There was at least a 57% decrease in weed biomass with the pre-emergence herbicide application and a post-emergence treatment or hoe weeding beyond 12 WAP compared to the application of pre-emergence herbicides and weeding alone for up to 12 WAP only. Our study observed a 60.7% increase in mango ginger rhizome yield when post-emergence weed control followed pre-emergence weed control compared to the pre-emergence herbicide application alone. Mango ginger is a perennial crop with initially slow growth and requires a weed-free period beyond 12 weeks after planting (WAP) to achieve acceptable weed control and optimum rhizome yield. Therefore, the pre-emergence application of oxyfluorfen at 0.24 kg a.iha^{-1} and post-emergence hoe weeding is recommended as this resulted in the best weed control and the highest mango ginger yield.

References

Bahadur, M.M., Azad, A.K. M., Hakim, M.A., Hossain, S.M.M., & Sikder, S.P. (2000). Effect of different spacing and potassium levels on the growth and yield of turmeric. *Pakistan Journal of Biological Sciences, 3* (4), 593-95. doi: 10.3923/pjbs.2000.593.595

Barla, S., Upasani, R.R., & Puran, A.N. (2015). Growth and yield of turmeric (Curcuma longa L.) under different weed management. *Journal Crop and Weed, 11* (Special Issue), 179-182.

Baruah, A., & Deka, J. (2020). Weed Management for Higher Productivity of Ginger (Zingiber officinale) in Plains of Assam. *Current Journal of Applied Science and Technology. 39* (31), 21-28. DOI: 10.9734/CJAST/2020/v39i3130985

Chandarana, H., Baluja, S., & Chanda, S.V. (2005). Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds. *Turkey Journal of Biology, 29*, 83-97.

Chatterjee, R., Chattopadhyay, P.K., Hnamte, V., Chongtham, T., & Datta, Ray, S.K. (2012). Assessment of Quality Characteristics of Mango Ginger (Curcuma amada Roxb.) Germplasm. *International Journal of Bio-resource and Stress Management, 3* (3), 380-382.

Chevallier, A. (1996). *The encyclopedia of medicinal plants.* London: Dorling Kindersley.

Divyashri, G., Krishna, Murthy, T.P., Sundareshan, S., Kamath, P., Murahari, M., Saraswathy, G.R., & Sadanandan, B. (2021). In silico approach towards the identification of potential inhibitors from Curcuma amada Roxb against H. pylori: ADMET screening and molecular docking studies. *BioImpacts, 11* (2), 119-127. doi: 10.34172/bi.2021.19

Eshetu, T., & Addisu, M. (2015). Effect of weed management methods on the growth and yield of ginger in Jimma, Ethiopia. *Journal of Biology, Agriculture and Healthcare, 5* (13), 200-204.

Farnsworth, D., Hamby, K.A., Bolda, M., Goodhue, R.E., Williams, J.C., & Zalom, F.G. (2017). Economic analysis of revenue losses and control costs associated with the spotted wing drosophila, Drosophila suzukii (Matsumura), in the California raspberry industry. *Pest Management Science. 73* (6), 1083-1090. doi: 10.1002/ps.4497

Huxley, A. (1992). *The new RHS dictionary of gardening.* MacMillan Press.

Hailemichael, G., & Tesfaye, K. (2008). The effects of seed rhizome size on the growth, yield and economic return of ginger (Zingiber officinale Rosc.). *Asian Journal of Plant Sciences 7* (2), 213-217. doi: 10.3923/ajps.2008.213.217

Hailemichael, G., & Tilahun, D. (2004). Current status of spice research. Progress report, IAR, Jimma Agricultural Research Center, Teppi Agricultural Research Sub-Center.

KAU (Kerala Agricultural University) (2006). Annual Report of the AICRP on weed control. Kerala Agricultural University, Vellanikkara, Thrissur.

Krishnamurthy, V.V., & Ayyaswamy, M. (2000). Role of herbicide on yield of turmeric. *Spice India, 13*, 9-11.

MoARD (2007). Annual yield assessment report 2007. Results on area, production and yield a coffee, tea and spices. Statistical Bullefin 150. Addis Ababa Ethiopia.

Nedunchezhiyan, M., Ravindran, C.S., & Ravi, V. (2013). Weed management in root and tuber crops in India: Critical analysis. *Journal of Root Crops 39* (2), 13-20

Nissankara, Rao, L.S., Kilari, E.K., & Kola, P.K. (2021). Protective effect of Curcuma amada acetone extract against high-fat and high-sugar diet-induced obesity and memory impairment. *An International Journal on Nutrition, Diet and Nervous System, 24*, 3. doi.org/10.1080/1028415X.2019.1616436

Osunleti, S.O., Olorunmaiye, P.M., Adeyemi, O.R., Asiribo, O.E., Lagoke, S.T.O., & Oni, O.E (2021a). Influence of organo-mineral ferilizer rates and weeding frequency on mango ginger (Curcuma amada Roxb.). *Acta fytotechn zootechn, 24,* (3), 206-211. https://doi.org/10.15414/afz.2021.24.03.206-211

Osunleti, S.O., Olorunmaiye, P.M., Adeyemi, O.R., & Osunleti, T.O. (2021b). Influence of different weed control methods on weed biomass, growth and yield of mango ginger (Curcuma amada Roxb.) in forest savannah transition agro-ecological zone of Nigeria. *Acta fytotechn zootechn 24,* (4), 272-278. https://doi.org/10.15414/afz.2021.24.04.272-278

Osunleti, S.O., Olorunmaiye, P.M., Adeyemi, O.R., Asiribo, O.E., & Lagoke, S.T.O. (2023). Growth and yield of mango ginger (Curcuma amada Roxb.) as influence of plant density, organo-mineral fertilizer, and weeding frequency. *Journal of Plant Nutrition, 46* (7), 1377-1390. DOI: 10.1080/01904167.2022.2067050

Osunleti, S.O., Olorunmaiye, P.M., & Adeyemi, O.R. (2022a). Influence of Different Weed Control Methods on Weed Density and Relative Importance Value of Weeds in Mango Ginger (Curcuma amada Roxb.). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 70* (1), 37-45. https://doi.org/10.11118/actaun.2022.004

Osunleti, S.O., Lagoke, S.T.O., Olorunmaiye, P.M., Adeyemi, O.R., Olatunde, O.E., Ajani, A.O., & Olaogun, O. (2022b). Profitability and Time Consumption as Influenced by Various Weed Control Methods in Curcuma Amada Roxb. *Agricultural Socio-Economics Journal, 22,* (3) 151-158. http://dx.doi.org/10.21776/ub.agrise.2022.022.3.1

Padalia, R.C., Verma, R.S., Sundaresan, V., Chauhan, A., Chanotiya, C.S., & Yadav, A. (2013). Volatile terpenoid compositions of leaf and rhizome of Curcuma amada Roxb. from Northern India. *Journal of Essential Oil Research, 25* (1), 17-22.

Padmapriya, K., Dutta, A., Chaudhuri, S., & Dutta, D. (2012). Microwave assisted extraction of mangiferin from Curcuma amada. *3 Biotech, 2*, 27-30. DOI 10.1007/s13205-011-0023-7

Ramachandran, C., Portalatin, G.M., Prado, A.M., Quirin, K., Escalon, E., & Melnick, S.J. (2017). In Vivo Antitumor Effect of Supercritical CO2 Extract of Mango Ginger (Curcuma amada Roxb) in U-87MG Human Glioblastoma Nude Mice Xenografts. *Journal of Evidence-Based Complementary and Alternaive Medicine, 22* (2), 260-267. doi: 10.1177/2156587216659390

Ramalingam, S., Chinnagounder, C., Perumal, M., & Palanisamy, M. (2013). Evaluation of New Formulation of Oxyfluorfen (23.5% EC) for Weed Control Efficacy and Bulb Yield in Onion. *American Journal of Plant Sciences, 4*, 890-895. http://dx.doi.org/10.4236/ajps.2013.44109

Salawudeen, A.A. (2017). Influence of organomineral fertilizer on the response of mango ginger (Curcuma amada Roxb.) to period of weed interference. M.Sc. thesis, Department of Plant Physiology and Crop Production., College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, Nigeria

Sah, D., Heisnam, P., Mahato, N.K., & Pandey, A.K. (2017). Weed Management in Ginger (Zingiber officinale Roscoe) through Integrated Approaches. *International Journal of Current Microbiology and Applied Sciences 6* (10), 1839-1845. doi:https://doi.org/10.20546/ijcmas.2017.610.22

Sasikumar, B. (2005). Genetic resources of Curcuma: diversity, characterization and utilization. *Plant Genetic Resources, 3* (2), 230-251.

Sathiyavani, E., & Prabhakaran, N.K. (2015). Effect of integrated weed management practices on plant height, number of tillers in turmeric during kharif season. *International Journal of Horticulture 5* (2), 1-8.

Singh, G., Singh, O.P., & Maurya, S. (2002). Chemical and biocidal investigations on essential oils of some Indian Curcuma species. *Progress in Crystal Growth and Characterization of Materials,* 45 (1-2), 75-81.

Sivakumar, V., Ravindrakumar, K., Chandrasekhar, C., Bhagavan, B.V.K., & Nirmal, Babu, K. (2019). Evaluation of pre and post emergence herbicides for effective weeds control in Ginger. *International Journal of Chemical Studies, 7* (4), 2597-2599.

Srivastava, A.K., Srivastava, S.K., & Shah, N.C. (2001). Constituents of the Rhizome Essential Oil of Curcuma amada Roxb. from India. *Journal of Essential Oil Research, 13* (1), 63-64.

Suresh, K., Rana, S.S., & Sharma, N. (2014). Weed management in turmeric. In: Biennial conference of Indian Society of Weed Science on Emerging challenges in weed management (pp. 105). Directorate of Weed Science Research, Jabalpur, Madhya Pradesh, India.

Tamta, A., Prakash, O., Punetha, H., & Pant, A. (2016). Chemical composition and in vitro antioxidant potential of essential oil and rhizome extracts of Curcuma amada Roxb. *Cogent Chemistry, 2* (1), 1168067

Unamma, C.P.A., Emyimmia, J., & Emezie, J.E. (1984). Critical period of weed interference in cocoyam/maize/sweret potato. *Tropical Pest Management, 31,* 21-23

Wauthoz, N, Balde, A, Balde, E.S., Damme, M.V., & Duez, P. (2007). Ethnopharmacology of Mangifera indica L bark and pharmacological studies of its main glucosylxanthone, mangiferin. *International Journal of Biomedical and Pharmaceutical Sciences, 1* (2), 112-119.

Received: October 14, 2023
Accepted: May 27, 2024

ODGOVOR MANGO ĐUMBIRA (*CURCUMA AMADA*) NA GUSTINU POPULACIJE I RAZLIČITE METODE SUZBIJANJA KOROVA U ZONI ŠUMO-SAVANE U JUGOZAPADNOJ NIGERIJI

Samuel O. Osunleti[1,2*] i Segun T.O. Lagoke[2]

[1]Department of Agricultural Technology, Edo State College of Agriculture and Natural Resources, Iguoriakhi, Edo State, Nigeria
[2]Department of Plant Physiology and Crop Production, Federal University of Agriculture, Abeokuta, Alabata, Abeokuta, Ogun State, Nigeria

R e z i m e

Poljski ogledi su sprovedeni u ranoj sezoni gajenja 2016. i 2017. godine na Nastavno-istraživačkom gazdinstvu Federalnog poljoprivrednog univerziteta, Abeokuta (07° 20’ N, 3° 23’ E, 159 m nadmorske visine) u zoni šumo-savane u jugozapadnoj Nigeriji, kako bi se procenio odgovor mango đumbira na gustinu populacije i različite metode suzbijanja korova. Tretmani su postavljeni po randomizovanom kompletnom blok dizajnu sa rasporedom podeljenih parcela, u tri ponavljanja. Glavni tretman činile su dve gustine populacije biljaka: 444.444 biljke/ha i 250.000 biljaka/ha, dok su pod-tretmani bili deset metoda suzbijanja korova. Prikupljeni podaci o rastu i prinosu biljke mango đumbira, kao i biomasi korova podvrgnuti su analizi varijanse (ANOVA), a srednje vrednosti tretmana su razdvojene primenom najmanje značajne razlike (LSD na nivou $p \leq 0,05$). Populacija mango đumbira u gustini od 444.444 biljke/ha rezultirala je povećanjem prinosa rizoma za 43,1% u poređenju sa gustinom od 250.000 biljaka/ha. Različite metode suzbijanja korova su dale značajno veći učinak u porastu useva, prinosu i komponentama prinosa u odnosu na zakorovljenu kontrolu. U odnosu na najvišu vrednost u obe godine, zakorovljenost na kontrolnoj varijanti rezultirala je smanjenjem prinosa rizoma za 91,4%. Došlo je do povećanja prinosa rizoma mango đumbira za 60,7% kada je suzbijanje korova nakon nicanja pratilo suzbijanje korova pre nicanja. Naše istraživanje je pokazalo da mango đumbir, kao višegodišnji usev sa početnim sporim rastom, zahteva period bez korova tokom prvih 12 nedelja nakon sadnje za prihvatljivo suzbijanje korova i optimalan prinos rizoma. Stoga se preporučuje primena oksifluorfena pre nicanja u dozi od 0,24 kg a.s./ha i okopavanje nakon nicanja.

Ključne reči: mango đumbir, rizomi, populacija, nakon nicanja, zakorovljenost.

Primljeno: 14. oktobra 2023.
Odobreno: 27. maja 2024.

[*]Autor za kontakt: e-mail: osunletis@gmail.com

Journal of Agricultural Sciences (Belgrade)
Vol. 69, No. 2, 2024
Pages 169-180

https://doi.org/10.2298/JAS2402169N
UDC: 634.233-152.6:575.17(55)
Original scientific paper

DETERMINATION OF S-ALLELES IN IRANIAN SOUR CHERRY (*PRUNUS CERASUS*) USING CONSENSUS PRIMERS

Samad A. Nazari[1*], Jafar Hajilou[2], Mehrshad Zeinalabedini[3] and Ali Imani[4]

[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran
[2]Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran
[3]Agriculture Biotechnology Research Institute of Iran (ABRII), Karaj, Iran
[4]Horticultural Departments of Seed and Plant Improvement Institute (SPII), Karaj, Iran

Abstract: Sour cherry is a tetraploid species, and gametophytic self-incompatibility (SI) operates in this species in the same way as in other stone fruit trees. However, while self-compatibility is most common in sour cherry and self-compatibility (SC) genotypes are rarely found, both SI and self-compatible (SC) types are selected in sour cherry. In this work, S-alleles have been identified for 70 sour cherry accessions and cultivars from the Shabestar regions of Iran, with S-genotypes of 68 cultivars identified for the first time. To identify the S-alleles, PCR-based methods were used. The amplification of the different alleles using combinations of the four forward primers (PaConsI-F, PruC2, PaConsII-F, EM-PC2consFD) and the five reverse primers (PruC4R, PCE-R, PaConsI-Rnew, PaConsII-R, EM-PC5consRD) revealed that they were the most useful for the identification of the sour cherry alleles. Nine known S-haplotypes (S6, S4, S9, S6m, S6m2, S24, S26, S35, S36a) were identified. In our study, alleles S6, S9, and S6m2 had a high frequency. It was shown that the consensus primers can be used to detect incompatibility alleles in sour cherry accessions. Our study has proved that the diversity of S alleles between the studied accessions was low, indicating low genetic diversity, which could also be due to the selection of superior genotypes by farmers.

Key words: sour cherry, self-incompatibility, S-RNase, consensus primer.

[*]Corresponding author: e-mail: Saliyoun66@gmail.com

Introduction

Gametophytic self-incompatibility (GSI) is a common genetic mechanism that promotes outcrossing in flowering plants. Most rosaceous fruit trees exhibit SI which is controlled by a single locus (S-locus) with multiple alleles (Franklin-Tong and Franklin, 2003). Sour cherry (2n = 4x = 32) is an allotetraploid species produced by hybridization of the diploid sweet cherry and tetraploid ground cherry (*Prunus fruticosa* Pall.), which displays gametophytic self-incompatibility (GSI), whereby the specificity of self-pollen rejection is controlled by alleles of the stylar and pollen specificity genes, S-RNase and SFB (S haplotype-specific F-box protein gene), respectively (Tsukamoto et al., 2006). Although most sour cherries are self-compatible (SC), some sour cherry selections are self-incompatible (SI), and therefore require a pollinator cultivar to achieve fruit set.

As each S-RNase allele has two introns varying in length, it was possible to differentiate S-RNase alleles by detecting intron size polymorphisms by DNA tests. Molecular methods based on PCR now allow precise S-genotyping and are being used to S-genotype many diverse sour cherry collections and wild populations (Lansari and Iezzoni, 1990; Yamane et al., 2001; Hauck et al., 2002; Yamane et al., 2003; Boskovic et al., 2006; Tsukamoto et al., 2006, 2008a, 2010; Khadivi-Khub et al., 2014; Lisek et al., 2015; Sebolt et al., 2017). To date, 34 S-haplotypes have been reported in sweet cherry and sour cherry, numbered S_1 to S_{37} as S_8, S_{11}, S_{15}, S_{23}, S_{24} and S_{25} were discontinued when they were later found to be identical to S_3, S_5, S_7, S_{14}, S_{22} and S_{21}, respectively. In sour cherry, 12 functional S-haplotypes (S_1, S_4, S_6, S_9, S_{12}, S_{13}, S_{14}, S_{16}, S_{26}, S_{33}, S_{34}, and S_{35}) were identified. Unlike sweet cherry, most sour cherry cultivars are SC due to the presence of either stylar-part mutants (designated Sm) or pollen-part mutants (designated S′) that disrupt either the stylar S-RNase or pollen SFB, respectively. So far, 9 non-functional S-haplotypes (S_1', S_{6m}, S_{6m2}, S_{13m}, $S_{13'}$, S_{36a}, S_{36b}, S_{36b2} and S_{36b3}) were identified in sour cherry (Hauck et al., 2006a, b; Tsukamoto et al., 2006, 2008a, 2010; Yamane et al., 2003; Sebolt et al., 2017). In fact, only non-functional variants were detected for S_{36}. As predicted by Hauck et al. (2006b), the presence of at least two of these non-functional S-haplotypes in the S-genotype results in SC (Yamane et al., 2001; Tsukamoto et al., 2006, 2010; Sebolt et al., 2017). The genetic switch from SI to SC in sour cherry results from the accumulation of non-functional S-haplotypes according to the one-allele-match model (Hauck et al., 2006a). In this model, the match between a functional pollen-S in the 2x pollen and its cognate functional S-Rnase in the style results in an incompatible reaction (Tsukamoto et al., 2008b). A similar reaction would occur regardless of whether the pollen contained a single functional pollen-S gene or two different pollen-S genes. The absence of any functional match results in a compatible reaction. Thus,

for successful self-fertilization, the 2x pollen must contain two non-functional S-haplotypes.

In this study, PCR analysis was performed using primers based on the conserved sequences of *S*-RNases to characterize the S-genotype of 70 sour cherry accessions and cultivars, including 68 accessions of Iranian sour cherry cultivars whose S-allele constitution had not been previously described.

Material and Methods

Plant material

Sixty-eight local Iranian sour cherry accessions from eight different regions of Shabestar, East Azerbaijan province, were used in this study (Figure 1, Table 1). In addition, two Hungarian sour cherry cultivars ('Cigany' $S_{6m}2S_9S_{26}S_{36b2}$; 'Erdi Botermo' $S_4S_{6m}S_{35}S_{36a}$) with known S-genotype were used as S-allele size standards.

Figure 1. Geographical locations of the collection sites of the sour cherry accessions.

Table 1. Sour cherry accessions analyzed by consensus primers and their S-genotypes identified in this study.

Origin	Accession name	S-genotyping with consensus primers		S-genotype
		1st intron	2nd intron	
Ali Beyglu	A01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A02	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A08	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A09	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A10	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A11	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A12	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A13	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A14	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A15	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A16	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A17	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A18	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A19	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Chehrgan	Ch01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch08	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch09	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch10	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch11	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Til	Ti01	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti08	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti09	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Nazarlou	N01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$

Continuation Table 1. Sour cherry accessions analyzed by consensus primers and their S-genotypes identified in this study.

Origin	Accession name	S-genotyping with consensus primers		S-genotype
		1st intron	2nd intron	
Qareh Tappeh	Q01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q05	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Tasuj	T01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Sharafkhaneh	Sh01	-	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Sis	S01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	S02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	S03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	S04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Hungarian cultivars	Cigany	S_9/S_{6m2}	$S_{6m2}/S_9/S_{36b2}/S_{26}$	$S_{6m2}S_9S_{26}S_{36b2}$
	Erdi botermo	S_{6m}	$S_{35}/S_{36a}/S_4$	$S_4S_{6m}S_{35}S_{36a}$

DNA isolation and consensus PCR analysis

Young, unfolded leaves were collected in the spring, frozen in liquid nitrogen, lyophilized, and stored at – 80ºC. Genomic DNA was extracted using the procedure described by Doyle and Doyle (1990). DNA concentrations and purification parameters were measured using a Picodrop 200 spectrophotometer (PicoDrop µl spectrophotometer, Cambridge, United Kingdom). PCR was conducted in a volume of 25 µL containing 50 ng of template DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3 mM MgCl2, 200 µM dNTP, 0.8 µM of each primer, and 0.625 units of Taq DNA polymerase. PCR reactions were set up on ice and the tubes were transferred to an Applied Biosystems thermal cycler (Life Technologies GmbH, Germany) and run for 4 min at 94ºC initial denaturation 10 cycles of 10 s at 94ºC, 2 min at the annealing temperature of each primer (see Table 2 for temperature) and 2 min at 68ºC, followed by 25 cycles of 10 s at 94ºC, 2 min at the annealing temperature and 2 min at 68ºC with 10 s per cycle to the 68ºC extension step. The PCR products were separated on a 1.3% agarose gel for about 2 h at 80

V. Also, for the first intron, PCR amplification was carried out for 4 min at 94ºC, 35 cycles of 1 min at 94ºC, 1 min at the annealing temperature of each primer (see Table 2 for temperature) and 1 min at 72ºC, with a 5-min final extension step at 72ºC. The products were run on a 2% agarose gel, for about 3 h at 70 V as described above. DNA bands were visualized by the ethidium bromide staining and observed and documented using the Geldoc G:box (Syngene, USA) gel imaging system. Fragment lengths were estimated by comparison with the 1 kbp DNA ladder. PCR products were purified using the NucleoSpin® gel and PCR clean-up kit (Macherey-Nagel, Germany) and sequenced by Macrogen Inc (Seoul, Korea). All PCR reactions and analyses were repeated at least twice.

Table 2. Nucleotide sequences of the consensus primers for PCR amplification of the first and the second intron of sour cherry S-RNases.

Primer	Sequence 5′ → 3′	Amplified region	Annealing temperature	References
PaConsI-F	(C/A)CTTGTTCTTG(C/G)TTT(T/C)GCTTTCTTC	1st intron	55 ºC	Sonneveld et al., 2003
PaConsI-R2 new	GCCATTGTTGCACAAATTGA			Sonneveld et al., 2006
Pru-C2	CTA TGG CCA AGT AAT TAT TCA AAC C	2nd intron	58 ºC	Tao et al., 1999
Pru-C4R	GGATGTGGTACGATTGAAGCG			
Pru-C2	CTA TGG CCA AGT AAT TAT TCA AAC C	2nd intron	58 ºC	Tao et al., 1999
PCE-R	TGTTTGTTCCATTCGCYTTCCC			
PaConsII-F	GGCCAAGTAATTATTCAAACC	2nd intron	58 ºC	Sonneveld et al., 2003
PaConsII-R	CA(T/A)AACAAA(A/G)TACCACTTCATGTAAC			
EM-PC2consFD	TCA-CMA-TYC-ATG-GCC-TAT-GG	2nd intron	55 ºC	Sutherland et al., 2004
EM-PC5consRD	CAA-AAT-ACC-ACT-TCA-TGT-AA-CAR-C			

Results and Discussion

As a result of the analyses, S-alleles were identified for the first time for 68 sour cherry accessions from the Shabestar regions. The amplification of the different alleles using combinations of the four forward primers (PaConsI-F, PruC2, PaConsII-F, EM-PC2consFD) and the five reverse primers (PruC4R, PCE-R, PaConsI-Rnew, PaConsII-R, EM-PC5consRD) proved to be the most useful for the identification of sour cherry alleles (Figure 2). In this study, the S-genotypes of all the 68 sour cherry accessions were analyzed (Table 1). Nine known S-haplotypes (S_4, S_6, S_9, S_{6m}, S_{6m2}, S_{24}, S_{26}, S_{35}, S_{36a}) previously reported in sour cherry were identified. ‘Cigany’ and ‘Erdi Botermo’ as the standard sour cherry cultivars showed S_4, S_{6m2}, S_9, S_{26}, S_{35} and $_{S36a}$ alleles, confirming the results of previous studies for these cultivars (Yamane et al., 2001; Yamane et al., 2003;

Hauck et al., 2006b; Tsukamoto et al., 2006, 2008a, 2010). In the present study, the S_6 and S_9 alleles of sour cherry could be easily distinguished in Iranian sour cherry according to other studies (Khadivi-Khub et al., 2014). Yamane et al. (2001) and Boskovic et al. (2006) also observed combinations of the S-RNases S_1, S_4, S_6, S_9 and S_{13} in sour cherry. Another study has also shown that the alleles S_1, S_2, S_3, S_4, S_5, S_6 and S_9 occurred with high frequency (Lacis et al., 2008; Ipek et al., 2011). S-allele frequency per region revealed that some S-alleles are more frequent in some regions and some only in a specific geographical area. It has also been confirmed that the Extremadura group of sour cherry has a higher frequency of alleles S_3 and S_6 (Wunsch and Hormaza, 2004), while the allele S22 is more frequent in the Mediterranean regions, as previously found in accessions from the Alicante region (Gisbert et al., 2008). The reason for the differences in the distribution of S-haplotypes in the different regions of Europe could be the common origin of the cultivars in isolated areas or the relationship between specific S-haplotypes with adaptive traits and the climatic conditions of the different areas (Cachi and Wünsch, 2014).

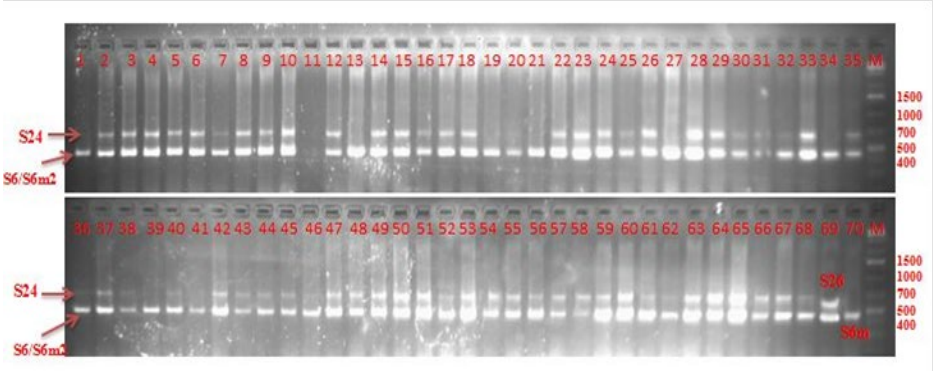

Figure 2. Amplification of intron II of the S-RNase gene in sour cherry accessions with the Pruc2 – PruC4R consensus primers. (M) 1 kb ladder, genotypes: 1 (Ch8), 2 (A12), 3 (T03), 4 (Q02), 5 (A11), 6 (N10), 7 (A16), 8 (Ti02), 9 (T07), 10 (A10), 11 (A15), 12 (Ch05), 13 (A03), 14 (A14), 15 (Q05), 16 (A17), 17 (T03), 18 (Ch12), 19 (Ch09), 20 (Ch01), 21 (Ch10), 22 (S02), 23 (S03), 24 (Ch04), 25 (Ch02), 26 (N09), 27 (Sh02), 28 (Ti08), 29 (N02), 30 (A13), 31 (Ti09), 32 (Ti02), 33 (Ti05), 34 (Ch6), 35 (A09), 36 (S01), 37 (A02), 38 (Ti04), 39 (Ch03), 40 (Ch07), 41 (T04), 42 (N03), 43 (N05), 44 (A07), 45 (N01), 46 (A04), 47 (N04), 48 (Q07), 49 (Ti10), 50 (T5), 51 (S04), 52 (A19), 53 (A01), 54 (Ti01), 55 (Q04), 56 (Sh01), 57 (Ti06), 58 (T01), 59 (A08), 60 (A05), 61 (Q06), 62 (A09), 63 (Q01), 64 (Sh03), 65 (Sh05), 67 (Q03), 68 (Sh04), 69 (Cigany), 70 (Erdi Botermo).

According to the obtained results, other methods should be used to distinguish among S_6-, S_{6m}- and S_{6m2}-RNases. For this purpose, PCR tests with the dCAPS marker (Derived Cleaved Amplified Polymorphic Sequences) and the insert-specific primer pair are required. The dCAPS markers can be used to distinguish S_{6m2}-Rnase from S_{6-} and S_{6m}-RNases. The PCR tests with the combination of dCAPS markers and insert-specific primer pairs are also required to distinguish among non-functional variants of the S_{36}-haplotype (Tsukamoto et al., 2010; Sebolt et al., 2017).

Polymerase chain reaction (PCR)-based S allele genotyping methods using consensus primers and separation of the products on agarose gels to detect length variations of the two introns have been reported (Tao et al., 1999, Wiersma et al., 2001, Sonneveld et al., 2003). Consensus primers will be especially useful for genotyping accessions of unknown parentage. As shown, consensus primers developed based on the sweet and sour cherry S-RNase sequences also lead to PCR amplification in a range of cherry species and could therefore be useful for studies of self-incompatibility in these species. S-allele identification by PCR analysis is highly reproducible and, thus, this technique can be used to complement other molecular methods in cherry cultivar identification (Wunsch and Hormaza, 2004; Khadivi-Khub et al., 2014; Lisek et al., 2015; Sebolt et al., 2017), almond (Channuntapipat et al., 2003; Tamura et al., 2000), apricot (Yaegaki et al., 2001; Hajilou et al., 2006, Muñoz-Sanz et al., 2017). The use of consensus primers enabled the identification of the majority of the S-alleles. However, the identification of some S-alleles was difficult because the resulting products were similar in size to those of other S-alleles, or no amplification products were present. To confirm and verify the results of the amplifications of intron I and intron II of the S-RNase gene, the S-RNase gene was purified and sequenced, verifying the results obtained with the consensus primers and unambiguously identifying the S-alleles in all the sour cherry genotypes tested.

However, in some accessions, only two alleles could be amplified, and some alleles could only be amplified with one of the primer pairs. One of the reasons for this is gaps or mismatches in the primer sequence, which can lead to inefficient annealing and, therefore, no amplification (Sonneveld et al., 2004; Cachi et al., 2017). Even if two alleles are very similar in size, the bands obtained after PCR amplification could be difficult to distinguish under standard agarose gel electrophoresis (Vaughan et al., 2006). This is the case for the S_6 and S_{24} alleles, which differ in 20 bp and cannot be differentiated after amplification with PruC2–PruC4R.

Knowledge of the S-genotypes of different accessions further enables the determination of relationships within the cultivated fruit species. The center of origin of cherry was described in Asia Minor including all of Transcaucasia, Iran and Turkmenistan. The diversity of the S-genotypes of the investigated cultivars

from parts of Turkey and Iran is lower than that of the European cherry genotypes. This could be due to the extensive fruit growing conditions and the absence of cherry breeding activities in the past. Furthermore, the number of studied cherry genotypes is lower compared to the data from Europe (Schuster, 2012).

Conclusion

Our study has proved that the diversity of S alleles between the studied accessions was low, indicating low genetic diversity, which could also be due to the selection of superior genotypes by farmers. Therefore, S-allele analysis is recommended for the local sour cherry cultivars of other regions of Iran, as well as for wild cherry, which is native to Iran. Our results demonstrate the limitation of using exclusively consensus primers for the reliable determination of S-genotypes.

Acknowledgements

We thank the Agriculture Biotechnology Research Institute of Iran and the Center of Excellence for Temperate Fruit Research in University of Tabriz for financial assistance.

References

Boskovic, R., Wolfram, B., Tobutt, K.R., Cerovic, R., & Sonneveld, T. (2006). Inheritance and interactions of incompatibility alleles in the tetraploid sour cherry. *Theoretical and Applied Genetics*, *112*, 315-326.

Cachi A.M., & Wünsch A. (2014). S-genotyping of sweet cherry varieties from Spain and S-locus diversity in Europe. *Euphytica*, *197*, 229-236.

Cachi, A.M., Wünsch, A., Vilanova, A., Guàrdia, M., Ciordia, M., & Aletà, N. (2017). S-locus diversity and cross-compatibility of wild *Prunus avium* for timber breeding. *Plant Breeding*, *136,* 126-131.

Channuntapipat, C., Wirthensohn, M., Ramesh, S.A., Batlle, I., & Arús, P. (2003). Identification of incompatibility genotypes in almond (Prunus dulcis Mill.) using specific primers based on the introns of the S-alleles. *Plant Breeding*, *122*, 164-168.

Doyle J.J., & Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *1,* 13-15.

Franklin-Tong, N., & Franklin, F.C.H. (2003). Gametophytic self-incompatibility inhibits pollen tube growth using different mechanisms*. Trends in Plant Science*, *8,* 598-605.

Gisbert, A.D., Badenes, M.L., Tobutt, K.R., Llacer, G., & Romero, C. (2008). Determination of the S-allele composition of sweet cherry (Prunus avium L.) cultivars grown in the southeast of Spain by PCR analysis. *The Journal of Horticultural Science and Biotechnology, 83,* 246-252.

Hajilou, J., Grigorian, V., Mohammadi, S.A., Nazemmieh, A., Romero, C., & Vilanova, L. (2006). Self- and cross-(in) compatibility between important apricot cultivars in northwest Iran. *The Journal of Horticultural Science and Biotechnology*, *81* (3), 513-517.

Hauck, N.R., Ikeda, K., Tao R., & Iezzoni, A.F. (2006a). The mutated S1-haplotype in sour cherry has an altered S-haplotype-specific F-box protein gene. *Journal of Heredity, 97* (5), 514-520.

Hauck, N.R., Yamane, H., Tao, R., & Iezzoni, A.F. (2006b). Accumulation of nonfunctional S-haplotypes results in the breakdown of gametophytic self-incompatibility in tetraploid Prunus. *Genetics, 172* (2), 1191-1198.

Hauck, N.R., Yamane, H., Tao, R., & Iezzoni A.F. (2002). Self-compatibility and incompatibility in tetraploid sour cherry (Prunus cerasus L.). *Sexual Plant Reproduction*, *15*, 39-46.

Ipek, A., Gulen, H., Akcay, M.E., Ipek, M., Ergin, S., & Eris, A. (2011). Determination of self-incompatibility groups of sweet cherry genotypes from Turkey. *Genetics and Molecular Research*, *10*, 253-260.

Khadivi-Khub, A., Zaman, Z., Fattahi, F., & Wünsch, A. (2014). S-allele diversity in Prunus L. Cerasus subgenus from Iran. *Biochemical Systematics and Ecology*, *53*, 1-7.

Lacis, G., Kaufmane, E., Rashal, I., Trajkovski, V., & Iezzoni, A.F. (2008). Identification of self-incompatibility (S) alleles in Latvian and Swedish sweet cherry genetic resources collections by PCR based typing. *Euphytica*, *160*, 155-163.

Lansari, A., & Iezzoni, A. (1990). A preliminary analysis of self-incompatibility in sour cherry. *Hortscience*, *25,* 1636-1638.

Lisek, A., Rozpara, E., Głowacka, A., Kucharska, D., & Zawadzka, M. (2015). Identification of S-genotypes of sweet cherry cultivars from Central and Eastern Europe. *Horticultural Science*, *4*, 13-21.

Muñoz-Sanz, J.V., Zuriaga, E., López, I., Badenes, M.L., & Romero, C. (2017). Self-(in) compatibility in apricot germplasm is controlled by two major loci, S and M. *BMC Plant Biology*, *17*, 82.

Schuster, M. (2012). Incompatible (S-) genotypes of sweet cherry cultivars *(Prunus avium* L.*)*. *Scientia Horticulturae*, *148*, 59-73.

Sebolt, A.M., Iezzoni, A.F., & Tsukamoto, T. (2017). S-genotyping of cultivars and breeding selections of sour cherry (*Prunus cerasus* L.) in the Michigan State University sour cherry breeding program. *Acta Horticulturae,* 1161.

Sonneveld, T., Robbins, T.P., & Tobutt, K.R. (2006). Improved discrimination of self-incompatibility S-RNase alleles in cherry and high throughput genotyping by automated sizing of first intron polymerase chain reaction products. *Plant Breeding*, *125*, 305-307.

Sonneveld, T., Tobutt, K.R., & Robbins, T.P. (2003). Allele-specific PCR detection of sweet cherry self-incompatibility (S) alleles S1 to S16 using consensus and allele-specific primers. *Theoretical and Applied Genetics, 107*, 1059-1070.

Sonneveld, T., Tobutt, K.R., Vaughan, S.P., & Robbins, T.P. (2004). Loss of Pollen-*S* Function in Two Self-Compatible Selections of *Prunus avium* Is Associated with Deletion/Mutation of an *S* Haplotype–Specific F-Box Gene. *Plant Cell, 17*, 37-51.

Sutherland, B.G., Robbins, T.P., & Tobutt, K.R. (2004). Primers amplifying a range of Prunus S-alleles. *Plant Breeding*, *123*, 582-584.

Tamura, M., Ushijima, K., Sassa, H., Hirano, H., & Tao, R. (2000). Identification of self-incompatibility genotypes of almond by allele-specific PCR analysis. *Theoretical and Applied Genetics*, *101*, 344-349.

Tao, R., Yamane, H., Sugiura, A., Murayama, H., Sassa, H., & Mori, H. (1999). Molecular Typing of S-Alleles Through Identification, Characterization and Cdna Cloning for S-Rnases in Sweet Cherry. *Journal of the American Society for Horticultural Science*, *124*, 224-233.

Tsukamoto, T., Hauck, N.R., Tao, R., Jiang, N., & Iezzoni, A.F. (2010). Molecular and genetic analyses of four nonfunctional S haplotype variants derived from a common ancestral S haplotype identified in sour cherry *(Prunus cerasus* L.). *Genetics, 184* (2), 411-427.

Tsukamoto, T., Hauck, N.R., Tao, R., Jiang, N., & Iezzoni, A.F. (2006). Molecular characterization of three non-functional S haplotypes in sour cherry (*Prunus cerasus*). *Plant Molecular Biology*, *62*, 371-383.

Tsukamoto, T., Tao, R., & Iezzoni, A.F. (2008a). PCR markers for mutated S-haplotypes enable discrimination between self-incompatible and self-compatible sour cherry selections. *Molecular Breeding*, *21* (1), 67-80.

Tsukamoto, T., Potter, D., Tao, R., Vieira, C.P., Vieira, J., & Iezzoni, A.F. (2008b). Genetic and molecular characterization of three novel S-haplotypes in sour cherry (Prunus cerasus L.). *Journal of Experimental Botany, 59* (11), 3169-85.

Vaughan, S.P., Russell, K., Sargent, D.J., & Tobutt, K.R. (2006). Isolation of S-locus F-box alleles in *Prunus avium* and their application in a novel method to determine self-incompatibility genotype. *Theoretical and Applied Genetics, 112*, 856-866.

Wiersma, P.A., Wu, Z., Zhou, L., Hampson, C., & Kappel, Z. (2001). Identification of new self-incompatibility alleles in sweet cherry (*Prunus avium* L.) and clarification of incompatibility groups by PCR and sequencing analysis. *Theoretical and Applied Genetics, 102*, 700-708.

Wunsch, A., & Hormaza, J.I. (2004). Molecular evaluation of genetic diversity and S-allele composition of Spanish local sweet cherry (*Prunus avium* L.) cultivars. *Genetic Resources and Crop Evolution, 51*, 635-641.

Yaegaki, H., Shimada, T., Moriguchi, T., Hayama, H., Haji, T., & Yamaguchi, M. (2001). Molecular characterization of S-RNase genes and S-genotypes in the Japanese apricot (*Prunus mume* Sieb. et Zucc.). *Sexual Plant Reproduction*, *13*, 251-257.

Yamane, H., Ikeda, K., Hauck, N.R., Iezzoni, A.F., & Tao, R. (2003) Self-incompatibility (S) locus region of the mutated S6-haplotype of sour cherry (*Prunus cerasus*) contains a functional pollen S allele and a non-functional pistil S allele. *Journal of Experimental Botany, 54* (392), 2431-2437.

Yamane, H., Tao, R., Sugiura, A., Hauck, N.R., & Iezzoni, A.F. (2001). Identification and characterization of S-RNases in tetraploid sour cherry (Prunus cerasus). *Journal of the American Society for Horticultural Science*, *126*, 661-667.

Received: February 11, 2023
Accepted: May 13, 2024

ODREĐIVANJE S-ALELA U IRANSKOJ VIŠNJI (*PRUNUS CERASUS*) POMOĆU KONSENZUSNIH PRAJMERA

Samad A. Nazari[1*], Jafar Hajilou[2], Mehrshad Zeinalabedini[3] i Ali Imani[4]

[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran
[2]Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran.
[3]Agriculture Biotechnology Research Institute of Iran (ABRII), Karaj, Iran
[4]Horticultural Departments of Seed and Plant Improvement Institute (SPII), Karaj, Iran

R e z i m e

Višnja je tetraploidna vrsta, koju karakterise gametofitna inkopatibilnost (SI) koja kod ove vrste deluje na isti način kao i kod ostalih koštičavih vrsta voćaka. Iako je samooplodnost (SO) najčešća kod višnje, auto-inkompatibilni (SI) genotipovi se retko nalaze, kod višnje su selekcionisani i SO i SI tipovi. U ovom radu, S-aleli su identifikovani za 70 genotipova i sorti višnje iz regiona Šabestara u Iranu, pri čemu je 68 genotipova identifikovano po prvi put. Za identifikaciju S-alela korišćene su metode zasnovane na lančanoj reakciji polimeraze. Amplifikacija različitih alela korišćenjem kombinacije četiri prednja prajmera (PaConsI-F, PruC2, PaConsII-F, EM-PC2consFD) i pet obrnutih prajmera (PruC4R, PCE-R, PaConsI-Rnew, PaConsII-R, EM-PC5consRD) je pokazala da su oni najkorisniji za identifikaciju alela višnje. Identifikovano je devet poznatih S-haplotipova (S6, S4, S9, S6m, S6m2, S24, S26, S35, S36a). U našem istraživanju aleli S6, S9 i S6m2 su imali visoku učestalost. Pokazalo se da se konsenzusni prajmeri mogu koristiti za detekciju alela inkompatibilnosti kod genotipova višnje. Naše istraživanje je dokazalo da je raznovrsnost S-alela među proučavanim genotipovima niska, ukazujući na nisku genetičku raznovrsnost, što takođe može biti rezultat odabira superiornih genotipova od strane proizvođača.

Ključne reči: višnja, auto-inkompatibilnost, S-RNaza, konsenzusni prajmer.

Primljeno: 11. februara 2023.
Odobreno: 13. maja 2024.

[*]Autor za kontakt: e-mail: Saliyoun66@gmail.com

Journal of Agricultural Sciences (Belgrade)
Vol. 69, No. 2, 2024
Pages 181-196

https://doi.org/10.2298/JAS2402181K
UDC: 632.4:635.656
632.4:632.937.1
Original scientific paper

BIOLOGICAL CONTROL OF FOOT AND ROOT ROT DISEASE OF PEA (*PISUM SATIVUM* L.) BY USING A FORMULATED PRODUCT OF *TRICHODERMA*

Fatima Karim[1*], Sk. Md. Mobarak Hossain[1], Md. Mahfuzul Hasan[2], Nayan Chandra Howlader[2] and Mohd Mujtaba Akib Bhuiyan[3]

[1]Department of Plant Pathology, Faculty of Agriculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh
[2]Department of Horticulture, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]MBBS, M Abdur Rahim Medical College, Dinajpur, Bangladesh

Abstract: Foot and root rot is one of the most serious yield-reducing diseases in peas. *Fusarium oxysporum* and *Sclerotium rolfsii* are primarily responsible for the development of pea foot and root rot diseases. This study was conducted to test the fungicide of the *Trichoderma* group for the control of foot and root rot in peas. Bio-fungicidal treatments of the *Trichoderma* group – Decoprima (T2), Lycomax (T3), Dynamic (T4), Tricost (T5), Provax 200 (T6), and *Trichoderma* (T7)– were used to compare results with untreated control plots. Lycomax (T3) performed well in suppressing pea foot and root rot disease, as well as other growth traits across different days after sowing (DAS). Lycomax (T3) gave the highest yield (39.81 g/plot) at 92 DAS compared to other treatments and untreated plots (11.67 g/plot). Although the chemical treatment Provax 200 (T6) controlled pea foot and root rot disease and yielded 33.76 g/plot, it is not eco-friendly. Lycomax (T3) achieved the greatest results at 75 DAS in all traits, including surviving seedlings (64.67/plot), infected plants (4/plot), plant height (67.33 cm/plot), and root branches per plant (33.33/plot). The plot treated with Lycomax (T3) had the greatest root length (28.33 cm/plot), root nodules (30.33/plant/plot), and branches (33.33/plant/plot) at 82 days after sowing. The flowers (76/plot) and pods (12.33/plot) peaked at 65 and 75 DAS, respectively. The current study has demonstrated that Lycomax (generic name: *Trichoderma)* is the best bio-fungicide to treat pea foot and root rot disease in an eco-friendly manner and boost production by improving plant health.

Key words: bio-fungicide, foot and root rot, *Fusarium*, pea, *Trichoderma*.

[*]Corresponding author: e-mail: dyutikarim24@gmail.com

Introduction

Legumes encompass peas, which produce pods containing seeds or beans. The peas that are commonly consumed include green, snow, and snap varieties. Garden peas typically have rounded, green pods with sweet and starchy peas inside. Peas belong to the Fabaceae family, also known as the bean or pulse family. This family, Leguminosae (Fabaceae), consists not only of peas such as *Pisum sativum* L., but also of nearly 18,000 other species. Additionally, it includes species such as *Lathyrus* (160), *Lens* (4), and *Vicia* (160–250).

Although peas originate from Asia and the Middle East, they are cultivated worldwide. This ancient crop, whose exact origins are uncertain but are believed to date back to around 1800 BC according to Rana et al. (2021), was initially discovered in regions around the Mediterranean, East Africa, Ethiopia, and Central Asia.

However, human intervention has led to its widespread cultivation in numerous temperate countries such as the Netherlands, France, the UK, the USA, Australia, and New Zealand. Pea cultivation has historical roots in Europe, dating back to the stone and bronze periods, while in India it has been cultivated since around 200 BC, and in New Zealand since 1900. Approximately 7000 years ago, peas were commonly intercropped with barley and wheat (McPhee, 2003).

Iqbal et al. (2019) observed notable disparities in the proximate composition, mineral content, and amino acid profile among lentils, chickpeas, cowpeas, and green peas. Peas are rich in vitamins, minerals, antioxidants, and phytonutrients that contribute to eye health and potentially mitigate certain malignancies. Specifically, peas contain lutein and zeaxanthin, which safeguard against cataracts and age-related macular degeneration (AMD) by blocking harmful blue light. Additionally, peas provide essential nutrients such as magnesium and potassium, which contribute to lowering blood pressure.

Despite their nutritional significance, peas are vulnerable to various diseases and infections caused by biological agents or climatic factors. Peas are known as a self-intolerant crop (Schreuder, 1949). The worst illness of *Pisum sativum* is foot and root rot among the destructive diseases. This condition is typically induced by a range of microorganisms, including bacteria, viruses, oomycetes, and fungi. Furthermore, root nematodes and other parasites exacerbate the plant damage, creating opportunities for fungal pathogens to proliferate.

Fungi predominantly contribute to foot and root rot disease in peas, with species such as *Fusarium solani* being particularly destructive. *Fusarium solani*, often associated with *Aphanomyces euteiches* Drechs, poses a significant threat to *Pisum sativum*, leading to stunted root systems and substantial crop losses. Common symptoms of foot and root rot include root tip browning, lesions, decay, leaf yellowing, wilting, and reduced crop yield.

Numerous research efforts have aimed to combat pea foot and root rot, albeit with limited success or potential hazards to health and the environment. Utilizing bio-fungicides such as *Trichoderma spp*. represents a promising approach due to their adaptability, rapid growth, and broad antibiotic spectrum. *Trichoderma spp*. effectively control soil-borne pathogens such as *Fusarium, Pythium, Phytophthora,* and *Rhizoctonia solani*, thereby promoting sustainable agriculture. These bio-fungicides not only reduce disease severity, but also enhance plant defence mechanisms and competitiveness against pathogens through the production of secondary metabolites such as chitinase, proteases, and β-1,3-glucanase. This research focused specifically on the use of fungicides of the *Trichoderma* group to develop an efficient and environmentally friendly strategy to control foot and root rot in peas.

Material and Methods

Planting material and experimental design

The investigation was conducted at the Plant Pathology Research Field of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh. The experimental field was composed of medium high granular loam soil. According to SRDI (Soil Resource Development Institute, Dinajpur), the pH of the soil was 5.2. Pea seeds sourced from a local supplier in Dinajpur were used for the experiment. A Randomized Complete Block Design (RCBD) with three replications was implemented, dividing the field into three blocks, each containing seven plots of 2 m x 1 m. The plots were arranged with a row spacing of 30 cm and a plant spacing of 6 cm, with 1 m between blocks and 0.5 m between plots. Seeds were sown at a depth of 3 cm. Seven treatments: T_2 = seed treatment with Decoprima and spraying at the basal region of the plants, T_3 = seed treatment with Lycomax and spraying at the basal region of the plants, T_4 = seed treatment with Dynamic and spraying at the basal region of the plants, T_5 = seed treatment with Tricost and spraying at the basal region of the plants, T_6 = seed treatment with Provax and spraying at the basal region of the plants, T_7 = seed treatment with *Trichoderma* and spraying at the basal region of the plants were evaluated with T_1 = control (without any treatment).

Soil preparation and fertilization

A well-drained area was selected and prepared meticulously for pea cultivation. The land was plowed, cross-plowed, and cleansed, and then harrowed and levelled to achieve the desired soil texture. According to BARI recommendations, manures and fertilizers were applied, including urea (250g/ha),

TSP (400g/ha), potash (200g/ha), boron (20g/ha), and zinc (20g/ha). Healthy, disease-free seedlings were chosen for sowing, and 250g of pea seeds were distributed per experimental allotment in rows. Sowing took place on 3 December 2021, with a row spacing of 30 cm and a sowing depth of 1–2 cm. During intercultural operations, germination commenced nine days after sowing, and two rounds of thinning were conducted at 15 and 30 days after sowing (DAS) to maintain an optimal plant population. Two irrigation treatments were applied, with the first irrigation at 30 DAS and the second after weeding, spaced 15 days apart. Weeding was performed twice, first at 30 DAS and then at 60 DAS, using a spade and a trowel. The bio-fungicidal solution was prepared by dissolving a specific quantity of bio-fungicide in the required amount of water. This solution was applied three times throughout the experiment. The spraying was carried out every fifteen days using a hand sprayer. The fungicidal solution was sprayed with appropriate care to ensure adequate coverage of the basal region of the plant. Before the spray tank was filled with the fungicide, it was thoroughly cleaned. Five bio-fungicides, one fungicide, and one control were selected as treatments for managing foot and root rot disease.

Table 1. Information of the bio-fungicides and fungicides.

Common name	Recommended dose	Formulation	Amount of fungicide/liter of water
Decoprima	*Trichoderma* sp. 4.35x 10^5 cfu/g *Streptomyces* sp. 1.16 x 10^6 cfu/g *Geobacillus* sp. 1.94 x 10^6 cfu/g	Powder	0.14 g
Lycomax	*Trichoderma harzianum* 2–3% *Trichoderma viridae* 0.5–1% *Metarhizium anisopliac* 2–3% *Beauveria bassiana* 2–3%	Powder	0.75 g
Dynamic wettable powders (WP)	*Bacillus amyloliquefaciens* 1x10^6 cfu/g	Powder	0.5 g
Tricost 1% WP	*Trichoderma* 2 x106 cfu/g	Powder	1 g
Provax 200 WP	Carboxin 37.50% + Thiram 37.50%	Powder	0.75 g
Trichoderma	*Trichoderma*	Powder	1 g

Data collection

Data on plant growth characteristics and diseases were gathered four times. The collection of data began at 45 DAS and proceeded every 10 days. The plots of pea were purposefully visited to measure the data of various agronomic parameters and to examine and record the data on foot and root rot diseases. After documenting infected plants, the dead plants were removed from the plot. Data was collected on some tested plant traits and disease incidence at different days after sowing (DAS).

Plants affected by *Fusarium* and *Sclerotium* show stunting, wilting, withering, chlorosis, necrosis, and defoliation of plant parts, which consequently results in the death of the whole plant. By observing the visual symptoms of foot and root rot, the number of defective or infected plants per plot was determined and recorded at various phases of plant growth. Approximately ten plants were randomly collected from each plot. Initial data collection began at 45 DAS and continued at 10-day intervals. In addition, the total number of infected plants per plot was counted. The incidence of disease was expressed as a percentage. The percent of disease incidence was calculated using the formula provided by Agrios (2005).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of inspected plants}} \times 100 \quad (1)$$

After evaluating the disease incidence in the experimental field, the plant samples showing the symptoms of foot and root rot were wrapped in paper bags and delivered to the Plant Pathology Laboratory of Hajee Mohammad Danesh Science and Technology University for isolation and confirmation of the fungal pathogens, namely *Fusarium oxysporum* and *Sclerotium rolfsii*.

Using a meter scale, the plant height was measured in centimeters at both the vegetative and reproductive development stages. For measuring plant height, 10 plants were randomly selected from each plot and data were recorded at 45, 55, 65, and 75 days after sowing (DAS). The number of branches on 10 plants from each plot was counted by randomly selecting 10 plants from each plot. Data were recorded at 45, 55, 65, and 75 DAS. At 77 and 82 DAS, the root length of 10 randomly selected plants from the interior rows of each plot was measured. At 77 and 82 DAS, the number of root branches was counted on 10 randomly selected plants from the interior rows of each plot. Ten plants were randomly selected from the interior rows of each plot in order to determine the number of root nodules. At 77 and 82 DAS, the total number of root nodules of the chosen plants was recorded. At 65 DAS, the number of flowers on 10 randomly selected plants was counted and recorded. As in the past, the plants were selected and the number of pods recorded at 75 DAS. When 80 to 90 percent of the plants had reached full maturity, the harvest was taken. Several indicators, such as pod color, pod filling, plant water content, etc., were used to determine the stage of maturation. Two harvests were conducted depending on maturity. Each time the seeds were harvested, they were hand-picked and packaged with appropriate identifiers based on the plot and treatment. After each collection, the weight of the pods and then the seeds were recorded immediately. The total output was calculated by adding the harvest from both times. Therefore, the total yield was recorded at 92 DAS. The total yield was measured in grams (g) for data preparation purposes.

Statistical analysis

A computer program, Statistix-10, conducted an accurate statistical analysis. The means of the interventions were compared using the LSD (Least Significant Difference) with an α value of 0.05% (Gomez and Gomez, 1984).

Results and Discussion

An attempt was made to observe the efficacy of the treatments on different parameters of the pea plants by collecting and comparing data of different dates of data collection.

Efficacy of the treatments

Fungal species, namely *Fusarium oxysporum* and *Sclerotium rolfsii*, were observed as pathogens of pea plants. The foot and root rot disease of *Vicia faba* can be caused by a number of different species of fungal pathogens, namely, *Fusarium*, *Rhizoctonia*, *Pythium*, and *Phoma* (Rubiales and Khazaei, 2022). At 45 DAS, the maximum number of survived plants was recorded at T_3 (86), followed by T_1, T_2, and T6. On the contrary, the minimum number of plants was recorded at T_4 (64), followed by T_5 and T_7 with 65 and 68.33 plants, respectively. At 55 DAS, the number of survived plants was highest at T_3 (86), followed by T_1 with 72 plants. The maximum number of survived plants at 65 DAS was at T_6 (72), followed by T_3 (71.67). The lowest number of survived plants was recorded at T_1 (55), followed by T_2 with 56.67 plants. At 75 DAS, the highest number of plants was recorded at T_6 (69.33), followed by T_2 and T_3 with 59.33 and 64.67 plants, respectively. In contrast, the lowest number of plants was recorded at T_1 (50), followed by T_7 with 53.33 plants (Table 2).

Table 2. Efficacy of treatments on survived seedlings or total plant population, and total number of infected plants per plot at different days after sowing (DAS).

Treatment	Number of survived seedlings				Number of infected plants			
	45 DAS	55 DAS	65 DAS	75 DAS	45 DAS	55 DAS	65 DAS	75 DAS
T1 = Control	72.00abc	72.00abc	55.00b	50.33c	5.00a	5.33bc	9.33a	11.00ab
T2 = Decoprima	74.00abc	74.00abc	56.67b	59.33abc	1.33bc	9.67a	7.33ab	12.67a
T3 = Lycomax	86.00a	86.00a	71.67a	64.67ab	0.00c	1.33c	2.00b	4.00c
T4 = Dynamic	64.00c	64.00c	60.33b	61.67abc	1.67bc	8.33ab	9.00a	10.33abc
T5 = Tricost	65.00bc	65.00bc	57.00b	59.00abc	2.00b	9.33ab	11.00a	12.33a
T6 = Provax	80.33ab	80.33ab	72.00a	69.33a	1.00bc	2.67c	2.33b	5.67bc
T7 = Trichoderma	68.33bc	68.33bc	55.33b	53.33bc	0.67bc	8.33ab	9.00a	11.00ab
%CV	7.86	3.51	2.17	3.44	31.09	3.82	3.83	2.17

Means having the same letter within a column do not differ significantly at the 5% level of probability.

After 45 DAS, some plants were found to be infected with various symptoms. At 45 DAS, the highest number of infected plants was at T_1 (5) and the lowest number of infected plants was at T_3 (0), followed by T_7 (0.67). At 55 DAS, the highest number of infected plants was recorded at T_2 (9.67), followed by T_4 with 8.33 plants. At 65 DAS, the highest number of infected plants was recorded at T_5 (11), followed by T_1 and T_2, with 9.33 and 7.33 plants, respectively. At 75 DAS, the highest number of infected plants was recorded at T_2 (12.67), followed by T_1 with 11 plants (Table 2). Different species of *Trichoderma* are used as biological control agents and as an alternative to chemical fungicides to control a variety of plant diseases (Hu et al., 2022).

The variation in the height of pea plants was observed due to application of different treatments (Table 3). At 45 DAS, plants reached the greatest height at T_6 (24.00 cm), whereas the height was the lowest at T_5 (13.00 cm). At 55 DAS, plants reached the highest height at T_3 (33.67 cm), followed by T_1 and T_6 with a plant height of 28 and 31.67 cm. The plants had the lowest height at T_2 (21 cm), followed by T_4 (23.33 cm). At 65 DAS, the plants were observed to have reached the highest height at T_3 (67.33 cm), followed by T_1 (58.67 cm) and T_2 (57.33 cm). At 75 DAS, the plants were observed to have reached the maximum height at T_3 (67.33 cm), followed by T_6 (65 cm). The plants with the lowest height were found at T_4 (47 cm), followed by T_1 (54.33 cm) and T_2 (51 cm). Although plant height increases with increasing plant age, variation was observed due to the implementation of several treatments. Maximum plant height was noted in the treated plots compared to the control plots. Plants treated with Lycomax (T3) reached the maximum height compared to other treatments at different dates of data collection.

Table 3. Efficacy of treatments on plant height and number of branches at different days after sowing (DAS).

Treatments	Plant height (cm)				Number of branches			
	45 DAS	55 DAS	65 DAS	75 DAS	45 DAS	55 DAS	65 DAS	75 DAS
T1 = Control	16.67ab	28.00abc	58.67ab	54.33b	9.00b	13.00abc	14.67b	20.67bc
T2 = Decoprima	18.67ab	21.00c	57.33ab	51.00b	6.67b	10.67bc	16.33b	19.00bc
T3 = Lycomax	20.67ab	33.67a	67.33a	67.33a	12.33a	17.67a	25.00a	33.33a
T4 = Dynamic	15.67b	23.33c	51.00b	47.00b	9.00b	11.00bc	16.00b	22.67b
T5 = Tricost	13.00b	23.33c	53.67ab	49.33b	8.67b	5.33d	10.33c	16.67c
T6 = Provax	24.00a	31.67ab	46.67b	65.00a	14.00a	14.00ab	22.00a	30.67a
T7 = Trichoderma	15.00b	24.67bc	48.33b	51.33b	8.67b	8.67cd	13.00bc	23.00b
%CV	2.17	2.44	7.86	6.42	3.82	2.22	2.42	3.83

Means having the same letter within a column do not differ significantly at the 5% level of probability.

The treatments applied had a great impact on the number of branches of the pea plants (Table 3). At 45 DAS, the highest number of branches was observed at T_6 (14), followed by T_3 (12.33). At 55 DAS, the plants obtained the highest number of branches at T_3 (17.67), followed by T_1 (13) and T_6 (14). At 65 DAS, the plants reached the maximum number of branches, recorded at T_3 (25), followed by T_6 (22). At 75 DAS, plants reached the highest number of branches at T_3 (33.33), followed by T_6 (30.67). It was observed that plants treated with bio-fungicides produced more branches than untreated plants. Among the treated bio-fungicides, Lycomax (T3) again showed the most effective performance in producing more branches in pea plants. Lycomax can reduce 9.09% of plant infection and 89.04% of leaf infection (Mollah and Hassan, 2023).

The plants with the highest number of flowers at T_3 (76) were followed by the plants treated with T_1 and T_6. The number of flowers was 50 and 69.67, respectively (Table 4).

The first fruiting was recorded at 75 DAS (Table 6). The highest number of pods was recorded at T_3 (12.33), followed by T_6 (11.67).

The lowest number of pods was recorded at T_1 (2.67), followed by T_2 (4.67), T_4 (4), and T_5 (3.65).

Table 4. Efficacy of treatments on flowering of plants (at 65 DAS), number of pods/plant (at 75 DAS), root length of plants/plot (at 77 and 82 DAS), and number of root branches of plants/plot (at 77 and 82 DAS).

Treatment	No. of flowers	No. of pods	Root length (cm)		No. of root branches	
	65 DAS	75 DAS	77 DAS	82 DAS	77 DAS	82 DAS
T1 = Control	50.00bc	2.67b	11.67bcd	20.00b	24.00c	20.00b
T2 = Decoprima	45.67c	4.67b	9.67cd	18.33bc	18.67e	22.33b
T3 = Lycomax	76.00a	12.33a	18.33a	28.33a	31.67a	33.33a
T4 = Dynamic	46.00c	4.00b	13.00bc	19.33b	23.67c	19.33b
T5 = Tricost	48.67c	3.67b	8.00d	13.33c	17.67e	16.33b
T6 = Provax	69.67ab	11.67a	15.67ab	27.33a	29.33b	33.67a
T7 = *Trichoderma*	49.33c	5.00b	10.67cd	20.67b	21.67d	20.33b
%CV	7.86	31.07	3.42	3.82	2.12	3.84

Means having the same letter within a column do not differ significantly at the 5% level of probability.

The root length of plants/plot was recorded at 77 and 82 DAS (Table 6). The impact of the applied treatments on the root length of the pea plants was significant in the study. At 77 DAS, the largest root length was recorded at T_3 (18.33 cm), followed by T_1 (11.67 cm) and T_4 (13). A small root length was observed at T_5 (8 cm), followed by T_2 (9.67 cm) and T_5 (8 cm). At 82 DAS, the plants had the highest root length at T_3 (28 cm), followed by T_1, T_4, T_6 and T_7 (Table 4). All the bio-control agents resulted in significantly better germination than the control

treatment and a significant rise in root and shoot length, leading to a high vigor index (Hoque et al., 2014). Plant height, branches, root length and number of root nodules increased with increasing age of the pea plants. *Trichoderma* species promote plant defense mechanisms and improve the growth of plant and root (Sharma et al., 2022).

The number of root branches/plant/plot was recorded at 77 and 82 DAS (Table 4). At 77 DAS, the number of plants with the maximum number of branches was highest at T_3 (31.67), followed by T_1 (24), and T_4 (23.67). At 82 DAS, the number of plants, with the lowest number of branches was at T_5 (16.33), followed by T_1, and T_2 (Table 4). Long bushy roots play a significant role in the final yield.

The average number of root nodules (plants/plot) was recorded at 77 and 82 DAS (Table 5). At 77 DAS, the maximum number of root nodules was noted at T_3 (23), followed by T_6 (22.33). The minimum number of root nodules was observed at T_1 (9.33), followed by T_2. At 82 DAS, the highest number of root nodules was noticed at T_3 (30.33), followed by T_4 (24.33). Root rot drastically reduces the number of roots available for symbiotic nodulation (Hwang et al., 1994). Thus, nodulation reduces as *Fusarium* root rot severity rises and contracted nodulation can slow plant growth. In addition, a 1% talc-based formulation of *T. harzianum* resulted in higher germination and improved plant height, root length and yield (Sinha et al., 2018).

Table 5. Efficacy of treatments on root nodules/plant/plot (at 77 and 82 DAS), pod weight/plant/plot (at 85 and 92 DAS), seed weight/plant/plot after harvest (at 85 and 92 DAS).

Treatment	Number of root nodules		Pod weight (g)		Seed weight (g)	
	77 DAS	82 DAS	85 DAS	97 DAS	85 DAS	92 DAS
T1 = Control	9.33c	19.33bc	0.49b	33.50c	0.23b	11.43b
T2 = Decoprima	10.67bc	14.67c	0.48b	28.31c	0.21b	14.86b
T3 = Lycomax	23.00a	30.33a	0.77a	76.46a	0.34a	39.47a
T4 = Dynamic	12.33bc	24.33ab	0.52b	33.64c	0.23b	13.14b
T5 = Tricost	13.33bc	17.33bc	0.52b	36.79bc	0.21b	19.99b
T6 = Provax	22.33a	27.33a	0.71a	69.05ab	0.31a	33.45a
T7 = *Trichoderma*	14.67b	20.00bc	0.47b	42.72abc	0.20b	18.04b
%CV	2.17	2.42	33.08	6.46	32.02	2.14

Means having the same letter within a column do not differ significantly at the 5% level of probability.

At 85 DAS, the highest pod weight was at T_3 (0.77 g), followed by T_6 (0.71 g). The lowest pod weight was recorded at T_7 (0.47 g), followed by T_1 with a pod weight of 0.49 g. At 92 DAS, the highest pod weight was obtained at T_3 (76 g), followed by T_6 and T_7 with a pod weight of 69.05 and 42.72 g, respectively (Table 5). The lowest pod weight was obtained at T_2 (28.31 g), followed by T_1, T_4 and T_5

with a pod weight of 33.50, 33.64 and 36.79 g, respectively. A good yield depends on the number of sufficient flowers and pods on the plants. Due to the application of bio-fungicides, the pea plants showed maximum yield. Of all the bio-fungicides used, Lycomax (T_3) performed best, as it produced more flowers and pods than before and thus played a significant role in yield. In the untreated control plot, the yield was lower. *Trichoderma harzianum* (Th3) significantly increases the numbers of pods/plant in the field (Ketta and Hewedy, 2021).

The seed weight of the different plots after harvest was recorded at different dates (85 DAS and 92 DAS) as shown in Table 5. At 85 DAS, the highest seed weight was recorded at T_3 (0.34 g), followed by T_6 (0.31 g). The lowest seed weight was recorded at T_7 (0.20 g), followed by T_1 (0.23).

At 92 DAS, the highest seed weight was recorded at T_3 (39.47 g), followed by T_6 (33.45 g). The lowest seed weight was recorded at T_1 (11.43 g), which was followed by T_2 (14.86 g), and T_4 (13 g). Provax-200 significantly reduces the seedling mortality and improves seed yield in pea (Akhter et al., 2015).

The total harvest (pod and seed weight) was recorded at 92 days after sowing (Table 6). The efficacy of the applied treatments was evaluated based on the total harvest (pod and seed weight). The maximum pod weight was obtained at T_3 (77.23 g), followed by T_6 (69.77 g). The minimum pod weight was recorded at T_2 (28.79 g), followed by T_1 (33.99 g).

Table 6. Total yield (pod and seed weight/plot) and disease incidence (%).

Treatments	Total yield		Disease incidence (%)			
	Pod weight (g)	Seed weight (g)	45 DAS	55 DAS	65 DAS	75 DAS
T1 = Control	33.99c	11.67b	10.31a	8.84ab	14.75a	20.06a
T2 = Decoprima	28.79c	15.07b	2.933bc	9.80 a	11.45ab	18.56a
T3 = Lycomax	77.23a	39.81a	0.00c	1.40c	1.94c	3.99c
T4 = Dynamic	34.16c	13.37b	4.64b	9.26ab	10.74ab	12.68ab
T5 = Tricost	37.13bc	20.20b	5.47b	11.34a	13.25a	16.19a
T6 = Provax	69.77ab	33.76a	4.30b	4.22bc	5.08bc	6.86bc
T7 = *Trichoderma*	43.19bc	18.24b	3.99b	7.94ab	9.93ab	12.77ab
%CV	2.16	6.83	28.64	29.33	3.84	2.69

Means having the same letter within a column do not differ significantly at the 5% level of probability.

The maximum seed weight was recorded at T_3 (39.81 g), followed by T_6 (33.76 g). If the seeds are treated with fungicides/botanicals, the severity of the disease decreases and the yield of lentil pods and plants increases (Shahiduzzaman, 2015). The minimum seed weight was recorded at T_1 (11.67 g), followed by T_2 with 15.07 g. The maximum number of plants was recorded in bio-fungicide treated plots compared to the control plots. A large number of plants eventually contributed to the yield. Application of *Trichoderma* can improve seedling growth

and yield in various cereals and vegetables such as tomato, cucumber, and maize (Hossain and Akhter, 2020). Different species of *Fusarium* are the most dominant species causing root rot in faba bean crops and are responsible for severe yield losses (Yu et al., 2023).

Disease incidence (%) during the plant growth period, at several days after sowing, was recorded on the basis of visible symptoms. Seven treatments were compared for disease incidence observed at 45 DAS, 55 DAS, 65 DAS and 75 DAS. At 45 DAS, the highest disease incidence (10.31%) was recorded at T_1 (control), whereas T_3 had the lowest disease incidence (0%), followed by T_2 (2.933%). The maximum disease incidence (11.34%) was observed at T_5 at 55 DAS, whereas T_3 had the minimum disease incidence (1.4%). The highest disease incidence (14.75%) was recorded at T_1 (control) at 65 DAS, whereas T_3 displayed the minimum disease incidence (1.94%). The final disease incidence was recorded at 75 DAS, with T_3 having the minimum disease incidence (3.99%) and T_1 having the highest (20.06%). Thus, the treatment with Lycomax (T_3) showed the best result in managing disease incidence (Table 6). *Trichoderma harzianum* treated pea seeds showed a disease incidence of 22.14% (Nazir et al., 2022). Among all bio-fungicides, Lycomax (T3) showed the most effective performance in plant protection. The chemical treatment such as Provax 200 also showed effective results in plant protection. By managing foot and root rot disease of pea, bio-fungicide (Lycomax) played the most important role and contributed significantly to the total yield. *Trichoderma* showed a great impact on plant survival in the present study. According to Kashem et al. (2011), the macerated extract of *Fusarium solani* + *Trichoderma harzianum* had the best result in combating root rot of lentil with the maximum seed germination (100%), and number of branches/five plants (15.56). The effectiveness of Trichoderma in disease suppression is attributed to several mechanisms, including hyper-parasitism, antibiosis, induced resistance in the host plant, and competition for nutrients and space (Harman et al., 2004). Studies have shown that *Trichoderma harzianum* exhibits antagonistic activities against various *Fusarium* species, including *Fusarium solani, F. oxysporum, and F. incarnatum*, with inhibition rates ranging from 50.8% to 85% (Hussein et al., 2022). The volatile compounds produced by *T. harzianum* contribute significantly to inhibiting *Fusarium* species. In pea crops, *Trichoderma* and its secondary metabolites play a crucial role in mitigating root rot disease by enhancing protective mechanisms (Ketta and Hewedy, 2021).

Regression coefficient between percent disease incidence and plant height

The linear regression analysis revealed a negative relationship between plant height and percent disease incidence. The response of plant height to the intensity of the percent disease incidence was estimated by the regression equation Y= -

0.875x + 46.07 (R^2 = 0.539). The fitted line plot graphically displayed the regression results with the equation between the dependent variable of plant height and the independent variable of percent disease incidence. The equation stated that plant height declined at a rate of 46.07 with an increase of one unit of percent disease incidence. The R^2 value of 0.539 indicated that 53% of the maximum plant height could be explained by the respective function (Figure 1).

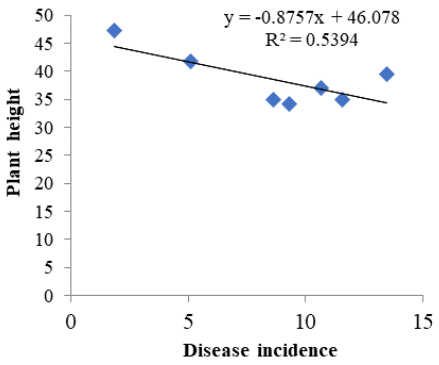

Figure 1. Regression co-efficient between percent disease incidence and plant height.

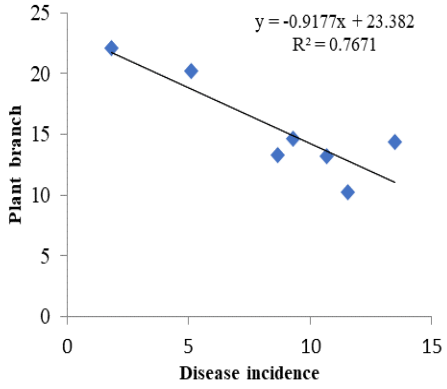

Figure 2. Regression co-efficient between percent disease incidence and branch number.

Regression coefficient between percent disease incidence and the number of plant branches

The linear regression analysis revealed a negative relationship between the number of plant branches and percent disease incidence. The response of the number of plant branches to the intensity of the percent disease incidence was determined by the regression equation Y = -0.917x + 23.38 (R^2 = 0.767). The fitted line plot graphically showed the regression results with the equation between the dependent variable of the number of plant branches and the independent variable of percent disease incidence. The equation indicated that the number of plant branches decreased at a rate of 23.38 (number) with an increase of one unit of percent disease incidence. The R^2 value of 0.767 indicated that 76% of the number of plant branches could be explained by the respective function (Figure 2).

Regression coefficient between percent disease incidence and plant root length

The linear regression analysis shows a negative relationship between root length and percent disease incidence. The response of root length to the intensity of

the percent disease incidence was determined by the regression equation Y= -0.470x + 12.44 (R^2 = 0.748). The fitted line plot graphically showed the regression results with the equation between the dependent variable of root length and the independent variable of percent disease incidence. The equation showed that the root length decreased at a rate of 12.44 (number) with an increase of one unit of percent disease incidence. The R^2 value of 0.748 indicated that 74% of the root length could be explained by the respective function (Figure 3).

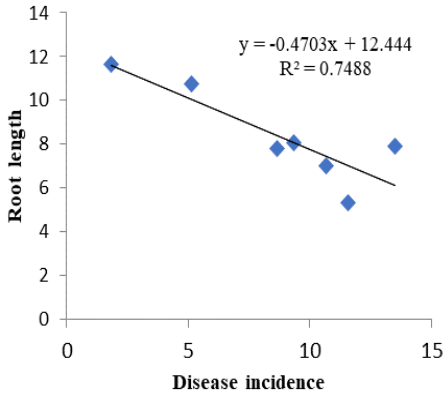

Figure 3. Regression coefficient between percent disease incidence and plant root.

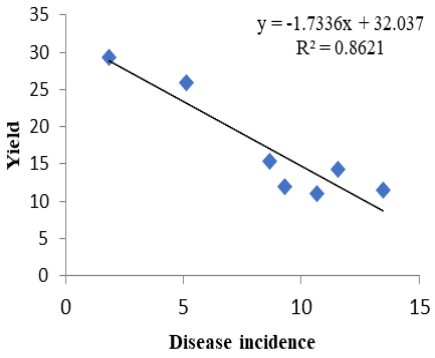

Figure 4. Regression coefficient between percent disease incidence and yield.

Regression coefficient between percent disease incidence and yield

The linear regression analysis revealed a negative relationship between yield and percent disease incidence. The response of yield to the intensity of the percent disease incidence was determined by the regression equation Y = -1.733x + 32.03 (R^2 = 0.862). The fitted line plot graphically represents the regression results with the equation between the dependent variable of total yield and the independent variable of percent disease incidence. The equation showed that the yield decreased at a rate of 32.03 (number) with an increase of one unit of percent disease incidence. The R^2 value of 0.862 indicated that 86% of the yield could be explained by the respective function (Figure 4).

Conclusion

The treatments had definite effects on total plant number, plant height, number of branches, root length, number of root nodules, flowering and fruiting of the pea

plants. The number of healthy plants was highest in the plot where the plants were treated with Lycomax. Lycomax-treated plants had more branches, greater height, longer roots, more root nodules and accelerated flowering and fruiting. The only chemical treatment (Provax 200) applied to the plants also showed healthy growth traits, but was found less effective than Lycomax. Poor growth traits of plants were found in the untreated control plot. Lycomax-treated plants were hardly affected by foot and root rot disease. Untreated plants, on the other hand, were mostly infected. Considering the eco-friendly management, Lycomax can be recommended for use in farmers' field for the feasible management of foot and root rot disease of pea and other vegetables.

Acknowledgements

The authors are thankful to Professor Dr. Sheikh Md. Mobarak Hossain, Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

References

Agrios, G.N. (2005). *Plant Pathology*. Florida (EUA).

Akhter, W., Bhuiyan, M.K.A., Sultana, F., & Hossain, M.M. (2015). Integrated effect of microbial antagonist, organic amendment and fungicide in controlling seedling mortality (*Rhizoctonia solani)* and improving yield in pea (*Pisum sativum* L.). *Comptes Rendus Biologies*, *338* (1), 21-28. https://doi.org/10.1016/j.crvi.2014.10.003.

Gomez, K.A., & Gomez, A.A. (1984). *Statistical procedures for agricultural research.* John Wiley and Sons.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, *2* (1), 43-56. https://doi.org/10.1038/nrmicro797.

Hoque, M.A., Hamim, I., Haque, M.R., Ali, M.A., & Ashrafuzzaman, M. (2014). Effect of some fungicides on foot and root rot of lentil. *Universal Journal of Plant Science*, *2* (2), 52-56. http://doi/org/10.13189/ujps.2014.020205.

Hu, Y.J., Yang, Yang, H.M., Jin, J., Yan, H.H., Wang J.P., & Zhang, R.Q. (2022). Synergistic activity of antagonistic *Trichoderma* spp. and *Rhizoctonia solani* increases disease severity on strawberry petioles. *European Journal of Plant Pathology*, *164*, 375-389. https://doi.org/10.1007/s10658-022-02568-w.

Husseina, N.A., Al-Janabi, H.J.K., Al-Mashhady, F.R., Abood Al-Janabi, J.K., & Shakir Al-Shujairi, A.R. (2022). Antagonistic activities of bioagent fungi *Trichoderma harzianum* and *Pleurotus ostreatus* against three species of Fusarium in cucumber plants. *Asia Pacific Journal of Molecular Biology and Biotechnology*, *30* (1), 12-21. http://doi/org/10.35118/apjmbb.2022.030.1.02.

Hossain, S., & Akter, F. (2020). Effects of Trichoderma-enriched biofertilizer and farmyard manure on the growth and yield of brinjal (*Solanum melongena* L.). *Dhaka University Journal of Biological Sciences*, *29* (1), 1-8. https://doi.org/10.3329/dujbs.v29i1.46525.

Hwang, S.F., Howard, R.J., Chang, K.F., Park, B., & Burnett, P.A. (1994). Etiology and severity of fusarium root rot of lentil in Alberta. *Canadian journal of plant pathology*, *16* (4), 295-303.

Iqbal, J., Yousaf, U., Zia, S., Asgher, A., Afzal, R., Ali, M., Sheikh, A.U.R., & Sher, A. (2019). Pulses Diseases "Important limiting factor in yield'' and their Managements. *Asian Journal of Research in Crop Science, 3* (2), 21.

Kashem, M.A., Hossain, I., & Hasna, M.K. (2011). Use of *Trichoderma* in biological control of foot and root rot of lentil (*Lens culinaris* Medik). *International Journal of Sustainable Crop Production, 6* (1), 29-35. http://doi/org/10.3329/bjar.v40i4.26945.

Ketta, H.A., & Hewedy, O.A.R. (2021). Biological control of *Phaseolus vulgaris* and *Pisum sativum* root rot disease using Trichoderma species. *Egyptian Journal of Biological Pest Control, 31*, 96. https://doi.org/10.1186/s41938-021-00441-2.

Mollah, M.M.I., & Hassan, N. (2023). Efficacy of *Trichoderma harzianum*, as a biological fungicide against fungal diseases of potato, late blight and early blight. *Journal of Natural Pesticide Research, 5*, 100047. https://doi.org/10.1016/j.napere.2023.100047.

McPhee, K. (2003). Dry pea production and breeding-a mini-review. *Journal of Food, Agriculture & Environment*, *1* (1), 64-69.

Nazir N., Badri Z.A., Bhat, N.A., Bhat, F.A., Sultan, P., Bhat, T.A., Rather, M.A., & Sakina, A. (2022). Effect of the combination of biological, chemical control and agronomic technique in integrated management pea root rot and its productivity. *Scientific Reports*, *12* (1), 11348. https://doi.org/10.1038/s41598-022-15580-1.

Rana, C., Sharma, A., Sharma, K.C., Mittal, P., Sinha, B.N., Sharma, V.K., & Rana, V. (2021). Stability analysis of garden pea (Pisum sativum L.) genotypes under North Western Himalayas using joint regression analysis and GGE biplots. *Genetic Resources and Crop Evolution*, *68,* 999-1010.

Rubiales, D., & Khazaei, H. (2022). Advances in disease and pest resistance in faba bean. *Theoretical and Applied Genetics*, *135*, 3735-3756. https://doi.org/10.1007/s00122-021-04022-7.

Schreuder, J.C. (1949). Voet- en vaatziekten bij erwten (pp. 136-143). *In: Tien jaar Peulvruchten Studie Combinatie (PSC).*

Shahiduzzaman, M.M.S. (2015). Efficacy of fungicides and botanicals in controlling foot and root rot of lentil. *Bangladesh Journal of Agricultural Research*, *40* (4), 711-715.

Sharma, A., Rani, M., Lata H., Thakur, A., Sharma, P., Kumar, P., Jayswal, D.K., & Rana, R.S. (2022). Global dimension of root rot complex in garden pea: Current status and breeding prospective. *Crop Protection*, *158*. https://doi.org/10.1016/j.cropro.2022.106004.

Sinha, A., Singh, R., & Verma, A. (2018). Bioefficacy of Trichoderma harzianum and Trichoderma viride against Fusarium oxysporum f. sp. capsici causing wilt disease in chilli. *Journal of Pharmacognosy and Phytochemistry*, *7* (5), 965-966.

Yu, H., Yang, F., Hu, C., Yang, X., Zheng, A., Wang, Y., Tang, Y., He, Y., & Meiyuan, Lv. (2023). Production status and research advancement on root rot disease of faba bean (*Vicia faba* L.) in China. *Frontiers in Plant Science, 14.* https://doi.org/10.3389/fpls.2023.1165658.

Received: September 4, 2023
Accepted: April 9, 2024

BIOLOŠKO SUZBIJENJE BOLESTI TRULEŽI STABLA I KORENA GRAŠKA (*PISUM SATIVUM* L.) KORIŠĆENJEM FORMULISANOG PROIZVODA SA GLJIVAMA IZ RODA *TRICHODERMA*

Fatima Karim[1*], Sk. Md. Mobarak Hossain[1], Md. Mahfuzul Hasan[2], Nayan Chandra Howlader[2] and Mohd Mujtaba Akib Bhuiyan[3]

[1]Department of Plant Pathology, Faculty of Agriculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh
[2]Department of Horticulture, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]MBBS, M Abdur Rahim Medical College, Dinajpur, Bangladesh

R e z i m e

Trulež stabla i korena je jedna od najozbiljnijih bolesti koje smanjuju prinos graška. *Fusarium oxysporum* i *Sclerotium rolfsii* su prvenstveno odgovorni za razvoj bolesti truleži stabla i korena graška. Ova studija je sprovedena kako bi se testirao fungicid na bazi gljivice *Trichoderma* za suzbijanje truleži stabla i korena graška. Bio-fungicidni tretmani na bazi ove gljivice – Decoprima (T2), Lycomax (T3), Dynamic (T4), Tricost (T5), Provax 200 (T6), i *Trichoderma* (T7) – korišćeni su za poređenje rezultata sa netretiranim kontrolnim parcelama. Lycomax (T3) je dao dobre rezultate u suzbijanju bolesti truleži stabla i korena graška, kao i u drugim osobinama rasta tokom različitih dana nakon setve. Lycomax (T3) je dao najviši prinos (39,81 g/parceli) 92 dana nakon setve u poređenju sa drugim tretmanima i netretiranim parcelama (11,67 g/parceli). Iako je hemijski tretman sa bio-fungicidom Provax 200 (T6) kontrolisao trulež stabla i korena graška i dao prinos od 33,76 g/parceli, on nije bezbedan za životnu sredinu. U tretmanu sa bio-fungicidom Lycomax (T3) postignuti su najbolji rezultati 75 dana nakon setve u pogledu svih karakteristika, uključujući preživele sejance (64,67cm/parceli), zaražene biljke (4/parceli), visinu biljke (67,33 cm/parceli) i grane korena po biljci (33,33/parceli). Na parceli koja je tretirana bio-fungicidom Lycomax (T3) postignuta je najveća dužina korena (28,33 cm/parceli), najveći broj nodula na korenu (30,33/biljci/parceli) i najveći broj grana (33,33/biljci/parceli) 82 dana nakon setve. Najviše cvetova (76/parceli) i mahuna (12,33/parceli) je bilo 65 odnosno 75 dana nakon setve. Ova studija je pokazala da je Lycomax (generički naziv: *Trichoderma*) najbolji bio-fungicid za tretiranje bolesti truleži stabla i korena graška i povećanje proizvodnje, a u isto vreme je bezbedan za životnu sredinu.

Ključne reči: bio-fungicid, trulež stabla i korena, *Fusarium*, grašak, *Trichoderma*.

Primljeno: 4. septembra 2023.
Odobreno: 9. aprila 2024.

[*]Autor za kontakt: e-mail: dyutikarim24@gmail.com

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2402197R
Vol. 69, No. 2, 2024 UDC: 582.736.3:581.4
Pages 197-206 Original scientific paper

CORRELATIONS BETWEEN MORPHO-AGRONOMIC TRAITS AND QUALITY COMPONENTS OF BIRDSFOOT TREFOIL

Vojo Ž. Radić[*], Ilija S. Komljenović and Borislav M. Petković

Faculty of Agriculture, University of Banjaluka, Bulevar Vojvode Petra Bojovića 1A 78000 Banjaluka, Bosnia and Herzegovina

Abstract: Birdsfoot trefoil is a perennial legume for the production of high-quality forage. Improving the production and quality of forage is one of the strategic objectives of breeding programs. The genotypes for this trial were selected from promising offspring collected from local populations in Bosnia and Herzegovina. A trial with eight genotypes (7 promising lines and 1 variety) was designed in a randomized block system with four replicates. In the first growth, 11 components of yield and quality of biomass were analyzed, and in the second growth, four additional parameters for seed production. In the first growth, highly significant correlations were found between plant height and the proportion of leaves (0.85**) and the yield of green matter and dry matter (0.81**), while a high negative correlation was found between the nitrogen-free extract (NFE) and the crude protein content (-0.79**). In the regrowth, statistically highly significant ($p<0.01$) positive correlations were found between the content of NFE and ash (0.77**). Statistically significant ($p<0.05$) positive relationships were found between green matter yield and dry matter yield (0.81**), green matter yield and stem diameter (0.79*), seed yield and number of pods (0.83*), and cellulose content and plant height (0.73*). The identification of positive correlations for certain productive and nutritional traits will be used in breeding programs for the creation of new varieties with improved forage quality.

Key words: birdsfoot trefoil, morphological traits, agronomic traits, correlations.

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.) is a very important perennial legume in the composition of grass-clover mixtures on natural and sown meadows in Bosnia and Herzegovina. It belongs to the group of perennial small-seeded legumes of high quality for the production of protein feed. It is usually sown in mixtures and only to a very limited extent as a pure crop (Radić, 2014). It tolerates a variety of

[*]Corresponding author: e-mail: vojo.radic@agro.unibl.org

soils and its particular speciality is that it tolerates a wide range of pH values from 4 to 9 (Marvin, 2004).

Its particular importance in Bosnia and Herzegovina lies in the possibility of cultivation on acidic soils. In their study, Vučković et al. (2007) came to the conclusion that the local populations of birdsfoot trefoil have several positive characteristics compared to other locations, based on the morphological, chemical and nutritional values investigated. As Sareen (2004) stated, the high proportion of easily digestible proteins is of particular importance for ruminants.

Knowledge of the correlations between the analyzed traits in the selection material is very important due to their interdependence, because changing one trait in the corresponding relationship also changes the others. Correlation coefficients are determined by correlations, regardless of what the dependent and independent variables are (Hallauer and Miranda, 1988). Genetic diversity is of crucial importance to breeders in the improvement of agricultural plant species. The complexity of the red clover genome with high heterozygosity and heterogeneity has been an obstacle in genomic analyses (Li et al., 2019).

Wróbel and Zielewicz (2019) investigated the variety of red clover and birdsfoot trefoil by taking samples of the green mass 9 times a week during a period from the end of April to the end of June. They carried out chemical analyses of the plant material in which they determined crude protein (CP), water-soluble carbohydrate (VSC), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin (ADL) and dry matter digestibility (DDM). The analyses showed that the birdsfoot trefoil leaf had a higher content of crude proteins, NDF and ADF than that of red clover at all stages of development.

Phenotypic differences in morpho-agronomic traits and forage quality traits within a population are based on genetic variation, but are also influenced by environmental conditions (precipitation, temperatures) and by the frequency of cutting, the maturity stage of the plant at the time of cutting and the cutting height (Swarup et al., 2021).

If there is a positive correlation between the two analyzed characteristics, selection of one characteristic also improves the other. Progress can be made in the selection of target traits through the direct selection of selected traits. However, negative correlations often mean problems with selection (Neyhart et al., 2019; Radinović et al., 2022).

The aim of this study is to evaluate the correlation between morphological and qualitative traits in perspective genotypes of the birdsfoot trefoil during a two-year experimental work. The results obtained can be used in selection based on the interdependence in the inheritance of the individual traits as well as in the simultaneous selection of several traits of birdsfoot trefoil.

Material and Methods

The genotypes for this trial were selected from promising offspring collected from local populations in Bosnia and Herzegovina. Seeds were collected from ten natural sites of birdsfoot trefoil in the mountains and hills and sown the following year. In the first selection cycle, seven promising accessions were selected from 28 new accessions.

The experiment was conducted southwest of Banja Luka (Fig. 1), in the village of Dobrnja on Manjača (N 44°39'57", E 17°00'24", 527 *m* above sea level). The trial was set up in four replicates in a randomized block system. The size of the trial plot was 1 x 2 *m*. The experimental units were sown manually at a row spacing of 0.2 *m*. The sowing rate was 1.5 $g\ m^{-1}$ or 15 $kg\ ha^{-1}$. No pesticides, chemical fertilizers or irrigation were used during the trial.

Figure 1. The experimental site at the "Center for Agriculture and Rural Development" Banja Luka, Bosnia and Herzegovina.

The results in 2013 showed the production characteristics of the newly created genotypes in comparison with the Tera variety and in comparison with each other. In 2013, there were two growths that were analyzed individually and together. The first growth was used for the production of green matter, and the second growth for the production of green matter and seeds. During the vegetation period, the following parameters were measured, analyzed and recalculated: green matter yield ($kg\ ha^{-1}$), dry matter yield ($kg\ ha^{-1}$), tiller diameter (*mm*), plant height (*cm*), number of tillers per plant, proportion of leaves (%), crude proteins, crude fats, crude cellulose, ash and nitrogen-free extract (NFE) in the first growth. In addition to these parameters, the following components of the seed yield were analyzed in the

regeneration: number of pods per plant, number of seeds in a pod, seed yield (*kg ha*$^{-1}$) and thousand-seed weight.

The chemical analyses were carried out according to the following methodology: crude proteins by the micro-Kjeldahl method, modified according to Bremner (1960), i.e., crude proteins by multiplication by a factor of 6.25; the amount of crude fats in the plant material by the Soxhlet method; the content of crude cellulose in the plant material by the Henneberg-Stohmanov method; the crude ash content in the plant material by annealing at 550°C to constant mass.

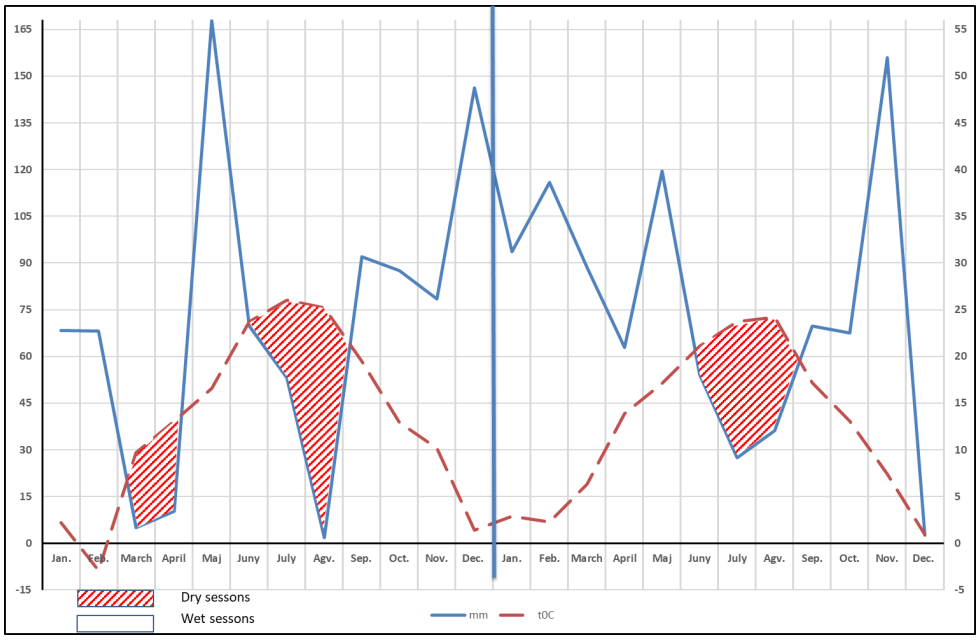

Figure 2. The relationship between precipitation and air temperature for the Banjaluka region in 2012 and 2013 (according to *H.Walther*).

Statistical analysis

The results of the biometric measurements were processed using PC applications for the Statistical Package for Social Sciences and Excel. The results of the analyzed traits were processed by analysis of variance (ANOVA) with a computer program using the GLM procedure. The Duncan's Multiple Range Test (DMRT) was used to determine the significance of the differences between the genotypes and their ranking at a significance level of p=0.01. Correlations between the analyzed traits were calculated as the Pearson's correlation coefficients and the significance was determined.

Results and Discussion

The positive tendency of plant breeding involves the improvement of several traits at the same time, but this is difficult to achieve due to the genetic correlations between the different traits (Breseghello and Coelho, 2013). The correct evaluation of genetic correlations requires large sample sizes and the presence of genetic generic data, which are not always available. Therefore, phenotypic correlations are often assumed to reflect genotypic correlations (Sodini et al., 2018). The correlations are caused by pleiotropic genes, physical linkage of genes on the chromosome or by the genetic structure of a population (Breseghello and Coelho, 2013).

The descriptive statistical parameters of the analyzed morphological-agronomic and qualitative traits of eight genotypes (seven promising offspring and the variety Tera) are listed in Table 1. Based on the f-test carried out, it can be seen that there was no statistical significance for fat content. There was a very high statistical significance for all other analyzed parameters ($p<0.001$). To determine which genotypes differed in the analyzed parameters, the Duncan's post-hoc test was used. The greatest differences were found in the tiller diameter and the leaf/stem ratio. The Duncan's test showed a higher degree of differentiation for quantitative than for qualitative traits.

Table 1. Average values of the analyzed characteristics of the first growth.

Genotype	1	2	3	4	5	6	7	8	f-test
Green mass	12.56^{a}	12.44^{a}	10.13^{b}	11.27^{b}	10.38^{b}	10.26^{b}	10.96^{b}	10.77^{b}	***
Dry mass	4.33^{b}	4.96^{a}	3.52^{d}	4.17^{bc}	$4.21b^{c}$	3.88^{c}	4.04^{bc}	4.06^{bc}	***
Stem diameter	1.53^{a}	1.47^{abc}	1.49^{ab}	1.53^{a}	1.35^{d}	1.38^{cd}	1.40^{bcd}	1.25^{e}	***
Stem height	37.2^{bc}	36.4^{bcd}	38.4^{b}	35.6^{cd}	35.9^{cd}	34.6^{d}	42.8^{a}	36.3^{bc}	***
Tiller number	7.65^{b}	6.65^{c}	9.15^{a}	7.15^{bc}	6.55^{c}	6.80^{c}	9.22^{a}	7.19^{bc}	***
Leaf/stem	65.5^{a}	59.5^{bcd}	56.5^{de}	55.5^{e}	62.0^{b}	61.5^{bc}	57.5^{de}	58.5^{cde}	***
Proteins	21.55^{a}	19.14^{b}	17.75^{b}	18.20^{b}	18.01^{b}	18.22^{b}	16.16^{c}	19.10^{b}	***
Fats	2.75^{a}	2.81^{a}	2.63^{a}	2.70^{a}	2.72^{a}	2.75^{a}	2.78^{a}	2.90^{a}	ns
Cellulose	22.95^{d}	23.42^{cd}	24.68^{ab}	23.96^{bc}	23.49^{cd}	23.86^{bc}	25.01^{a}	23.52^{cd}	***
Ash	9.55^{b}	10.44^{b}	10.64^{b}	11.61^{a}	11.46^{a}	10.44^{b}	10.23^{b}	11.44^{a}	***
NFE	43.2^{b}	44.19^{bc}	44.30^{bc}	43.53^{b}	44.32^{bc}	44.73^{bc}	45.82^{a}	43.04^{b}	**

**** ($p<0.001$), *** ($p<0.01$), * ($p<0.05$), ns – no significant, $^{a,b,c,d...}$ Values denoted by the same letter are not significantly different at the $p<0.01$ level of probability (Duncan's Multiple Range Test), NFE – nitrogen-free extract.

For regrowth, the components of yield and quality of green matter and the components of seed yield were analyzed (Table 2). The F-test shows that there was no statistical significance for thousand-seed weight, protein content and NFE. A

low significance ($p<0.05$) was found for the number of tillers per plant. A very high difference ($p<0.01$) was found between green matter yield, plant height and cellulose and ash content. A very high ($p<0.001$) difference was found for all other analyzed characteristics.

The Duncan's test was used to determine the difference between the genotypes within each trait. The greatest difference was noted in the number of pods per plant and the seed yield. There is a very high degree of differentiation in the ratio of leaf to stem among the genotypes tested.

Table 2. Average values of the investigated characteristics of regrowth.

	1	2	3	4	5	6	7	8	F- test
Green matter	10.37^{a}	9.42^{b}	9.73^{ab}	9.56^{ab}	9.34^{b}	9.2^{b}	9.82^{ab}	9.26^{b}	**
Dry mass	2.77^{a}	2.46^{bc}	2.47^{bc}	2.41^{bc}	2.59^{abc}	2.43^{bc}	2.63^{ab}	2.38^{c}	***
Stem diameter	1.47^{a}	1.35^{ab}	1.41^{a}	1.42^{a}	1.11^{c}	1.14^{c}	1.38^{ab}	1.22^{bc}	***
Plant height	31^{ab}	28.2^{ab}	28.4^{ab}	25^{b}	24.6^{b}	27.6^{b}	34.8^{a}	25^{b}	**
Tiller number	8.24^{ab}	7.23^{ab}	9.21^{ab}	7.65^{ab}	7.13^{ab}	6.98^{b}	9.57^{a}	8.11^{ab}	*
Seeds per pod	10.8^{b}	11.2^{b}	18.8^{a}	20.2^{a}	12.8^{ab}	11.0^{b}	14.6^{b}	14.8^{b}	***
Pods per plant	12.8^{e}	21.3^{bc}	22.5^{b}	18.8^{cd}	25.3^{a}	26.5^{a}	17.8^{d}	20.4^{bcd}	***
Seed weight	1.15^{a}	1.15^{a}	1.15^{a}	1.25^{a}	1.20^{a}	1.30^{a}	1.25^{a}	1.30^{a}	ns
Grain yield	195^{e}	236.5^{d}	245.0^{c}	268.5^{b}	280.5^{a}	27.05^{a}	243.0^{cd}	241.5^{cd}	***
% leaf	58.5^{a}	48.2^{d}	42.7^{e}	49.9^{cd}	52.4^{bc}	51.1^{cd}	55.1^{ab}	53.2^{bc}	***
Proteins	17.5^{a}	18.51^{a}	18.86^{a}	16.59^{a}	16.83^{a}	17.06^{a}	16.48^{a}	18.55^{a}	ns
Fat	2.85^{a}	2.89^{a}	2.74^{ab}	2.52^{aba}	2.90^{a}	2.68^{ab}	2.56^{b}	2.65^{ab}	***
Cellulose	28.96^{ab}	27.40^{b}	28.55^{ab}	28.6^{ab}	28.2^{ab}	29.4^{ab}	30.5^{a}	27.05^{b}	**
Ash	11.23^{ab}	9.71^{b}	12.1^{a}	11.68^{a}	11.4^{ab}	11.2^{ab}	12.5^{a}	12.47^{a}	**
NFE	39.46^{a}	41.49^{a}	37.75^{a}	40.61^{a}	40.67^{a}	39.66^{a}	37.96^{a}	39.28^{a}	ns

**** ($p<0.001$), *** ($p<0.01$), * ($p<0.05$), ns – no significant, $^{a,b,c,d...}$ Values denoted by the same letter are not significantly different at the $p<0.01$ level of probability (*Duncan's Multiple Range Test*), NFE – nitrogen-free extract.

Table 3 shows the correlation coefficients between the 11 characteristics analyzed in the first cut. Highly significant correlations were found between height and leaf percentage (0.85**) and green and dry matter yield (0.81**). Vasiljević et al. (2006) found high correlations between the yield of green matter and dry matter and plant height in their studies on red clover, assuming that we can increase the production of green matter by selecting taller plants.

The coefficients of the qualitative traits showed a high significance level of the relationship between NFE and leaf content, while a high negative correlation was found between NFE and crude protein content (-0.79**). The proportion of crude ash was highly correlated with the number of tillers (0.78**), while a negative correlation was found with the proportion of leaves (-0.71*) and the crude protein content.

Table 3. Correlation coefficients between the analyzed properties of the first cut.

	11	10	9	8	7	6	5	4	3	2
10	-0.25									
9	**0.73***	0.04								
8	-0.21	0.05	-0.35							
7	**-0.79***	-0.34	**-0.87****	0.21						
6	-0.21	-0.53	**-0.71***	0.15	0.68					
5	0.44	-0.36	**0.78***	-0.33	-0.42	-0.39				
4	0.61	-0.38	0.69	-0.27	-0.48	-0.27	**0.85****			
3	-0.06	-0.41	0.02	-0.64	0.26	-0.17	0.22	0.06		
2	-0.18	-0.13	-0.59	0.45	0.40	0.30	-0.56	-0.18	0.15	
1	-0.35	-0.49	-0.55	0.30	0.66	0.37	-0.20	0.01	0.50	**0.81****

p<0.05*; p<0.01**; N=8; Properties: 1– green matter, 2 – dry matter yield, 3 – stem diameter, 4 – plant height, 5 – number of tillers, 6 – leaf share, 7 – crude protein, 8 – crude fat, 9 – crude cellulose, 10 – ash, 11 – NFE.

The correlation coefficients of the analyzed traits in the regrowth between 15 traits, based on the average values of all traits, are shown in Table 4. The obtained values of the correlation coefficients show that most of the qualitative traits had a positive relationship with each other, except for the proportion of leaves, which had a negative relationship with most of the measured parameters. The relationships between the qualitative characteristics were mostly negative.

Table 4. Correlation coefficients between the analyzed properties of the second cut.

	15	14	13	12	11	10	9	8	7	6	5	4	3	2
14	**0.77***													
13	-0.52	0.23												
12	0.40	-0.65	-0.41											
11	-0.12	-0.16	-0.67	0.37										
10	0.05	0.13	0.29	-0.004	-0.51									
9	0.23	0.08	0.02	-0.25	-0.38	-0.39								
8	-0.09	0.47	0.14	-0.68	-0.37	0.23	0.50							
7	0.18	-0.13	-0.18	0.13	0.06	-0.55	**0.83***	0.30						
6	-0.31	0.54	0.00	-0.62	0.01	-0.52	0.27	0.09	-0.04					
5	**-0.87****	0.66	0.44	-0.39	0.11	-0.01	-0.43	-0.15	-0.47	0.42				
4	-0.55	0.13	**0.73***	-0.12	-0.18	0.33	-0.54	-0.22	-0.51	-0.23	0.67			
3	-0.26	0.02	0.21	-0.19	0.13	-0.02	-0.68	-0.52	**-0.80***	0.36	0.58	0.51		
2	-0.19	-0.02	0.48	0.38	-0.33	0.64	-0.57	-0.48	-0.58	-0.42	0.30	0.62	0.33	
1	-0.36	0.09	0.41	0.10	-0.08	0.40	**-0.79***	-0.57	-0.84	-0.02	0.55	0.64	**0.79***	**0.81***

p<0.05*; p<0.01**; N=8; Properties: 1– green matter, 2 – dry matter yield, 3 – stem diameter, 4 – plant height, 5 – number of stems, 6 – number of seeds per pod, 7 – number of pods, 8 – seed weight, 9 – grain yield,10 – leaf share, 11 – crude protein, 12 – crude fat, 13 – crude cellulose, 14 – ash, 15 – NFE.

Negative correlations were found between protein content and yield of green matter, dry matter, plant height, cellulose content, ash and NFE. Crude cellulose content was negatively correlated with protein, fat and NFE content, while it was positively correlated with ash content.

Statistically highly significant positive correlations with a significance level of $p<0.01$ were observed between NFE content and ash (0.77**). Statistically significant ($p<0.05$) positive correlations were found between green matter yield and dry matter yield (0.81**), green matter yield and stem diameter (0.79*), seed yield and number of pods (0.83*), cellulose content and plant height (0.73*) and the proportion of NE and ash (0.77*).

A highly significant negative correlation was found between NFE and the number of stems (-0.87**) and a significantly negative correlation between green matter yield and seed yield (-0.79*), stem diameter and number of pods and NFE content and number of tillers (-0.87**). Genotypes with higher forage production had a lower seed yield (Radić et al., 2011).

Conclusion

Positive correlations were found between yield and the yield components, green matter and seeds. These values indicate the possibility of improving yield by using individual components such as selection criteria in the breeding process.

In the newly selected synthetics, a significant correlation was found between the number of pods and seed yield (0.83*) and between plant height and the percentage of crude cellulose (0.73*). A negative correlation was found between green matter yield and seed yield (-0.79*) and between the number of tillers and NFE (-0.87**).

Potential genotypes such as 1, 2 and 4 have been identified as useful for improved production of productive and nutritional traits of birdsfoot trefoil, while genotypes 5 and 6 are of particular importance for future breeding programs due to their good generative properties.

References

Bremner, J. (1960). Determination of nitrogen in soil by the Kjeldahl method. *Journal of Agricultural Sci*ences, *55*, 1-23.

Breseghello, F., & Coelho, A.S.G. (2013). Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). *Journal of Agricultural and Food Chemistry*, *61*, 8277-8286.

Hallauer, A.R., & Miranda, J.B. (1988). *Quantitative genetics in maize breeding.* Iowa State University Press. Ames. Iowa. USA.

Li, W., Riday, H., Riehle, C., Edwards, A., & Dinkins, R. (2019). Identification of single nucleotide polymorphism in red clover (*Trifolium pratense* L.) 2019 using targeted genomic amplicon sequencing and RNA-seq. *Frontiers in Plant Science, 10*, 1257. doi: 10.3389/fpls.2019.01257

Marvin, V. Hall (2004). htt://www.forages.psu.edu.

Neyhart, J.L., Lorenz, A.J., & Smith, K.P. (2019). Multi-trait improvement by predicting genetic correlations in breeding crosses. *G3: Genes. Genomes. Genetics, 9* (10), 3153-3165.

Radinović, I., Vasiljević S., & Branković, G. (2022). Correlations of morpho-agronomic traits and forage quality properties in diverse red clover (*Trifolium pratense* L.) collections. *Journal of Agricultural Sciences (Belgrade), 67* (2), 139-151.

Radić, V., Gatarić, Đ., & Đurić, B. (2011). Correlation between the yield and the yield components of *Lotus corniculatus* L. genotypes. 46. hrvatski i 6. međunarodni simpozij agronoma (pp. 473-476). Opatija. Hrvatska.

Radić, V. (2014). Karakterizacija i ocjena oplemenjivačke vrijednosti lokalnih populacija (*Lotus corniculatus* L.) iz Bosne i Hercegovine. Univerzitet u Beogradu, Poljoprivredni fakultet Beograd-Zemun.

Sareen, S. (2004). Seed production potential in birdsfoot trefoil (*Lotus corniculatus* L.*)*. *Lotus Newsletter*, 34, 5-11.

Sodini, S.M., Kemper, K.E., Wray, N.R., & Trzaskowski, M. (2018). Comparison of genotypic and phenotypic correlations: Cheverud's conjecture in humans. *Genetics, 209,* 941-948.

Swarup, S., Cargill, E., Flagel, L., Kniskern. J., & Glenn, K.C. (2021). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Science*, *61* (2), 839-852.

Vasiljević, S., Šurlan-Momirović, G., Živanović, T., Ivanović, M., Mihailović, V., Mikić, A., Katić, S., & Milić, D. (2006). Genetic analysis of inheritance and mutual relationships among yield components, morphological-biological traits and yield of green mass of red clover (*Trifolium pratense* L.). *Genetika, 38* (1), 1-8.

Vučković, S., Stojanović, I., Prodanović, S., Ćupina, B., Živanović, T., Vojin, S., Jelačić, S. (2007). Morphological and nutritional properties of birdsfoot trefoil (*Lotus corniculatus* L.) autochthonus populations in Serbia and Bosnia and Herzegovina. *Genetic Resources and Crop Evolution, 54*, 421-428.

Wróbel, B., & Zielewicz, W. (2019). Nutritional value of red clover (*Trifolium pratense* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) harvested in different maturity stages. *Journal of Research and Applications in Agricultural Engineering*, *64* (4), 14-19.

Received: November 14, 2023
Accepted: June 6, 2024

KORELACIJE MORFOLOŠKO-AGRONOMSKIH OSOBINA I KOMPONENTI KVALITETA SMILJKITE

Vojo Ž. Radić*, Ilija S. Komljenović i Borislav M. Petković

Poljoprivredni fakultet, Univerzitet u Banja Luci, Bulevar Vojvode Petra Bojovića 1A 78000 Banja Luka, Bosna and Hercegovina

R e z i m e

Smiljkita je višegodišnja mahunarka za proizvodnju visokokvalitetne kabaste stočne hrane. Poboljšanje produkcije i kvaliteta stočne hrane jedan je od strateških ciljeva u oplemenjivačkim programima. Genotipovi za ovaj ogled odabrani su od perspektivnih potomstava koji su prikupljeni iz lokalnih populacija na području Bosne i Hercegovine. Ogled sa osam genotipova (7 perspektivnih linija i 1 sorta) postavljen je po slučajnom blok sistemu u četiri ponavljanja. U prvom porastu analizirano je 11 komponenti prinosa i kvaliteta zelene mase, a u drugom još četiri parametra za produkciju sjemena. Utvrđeni su Pirsonovi koeficijenti korelacija. U prvom porastu utvrđene su visokoznačajne korelativne veze između visine biljke i udjela lista (0,85**) kao i prinosa zelene mase i suve materije (0,81**), dok je visoka negativna korelativna veza utvrđena između *BEM*-a i sadržaja sirovih proteina (-0,79**). U drugom porastu uočene su statistički visoko značajne ($p<0,01$) pozitivne korelativne veze između sadržaja *BEM*-a i pepela (0,77**). Značajne statistički ($p<0,05$) pozitivne veze su konstatovane između prinosa zelene mase i prinosa suve materije (0,81**), prinosa zelene mase i debljine stabljike (0,79*), prinosa sjemena i broja mahuna (0,83*) i sadržaja celuloze i visine biljke (0,73*). Identifikacija pozitivnih korelativnih veza za pojedine produktivne osobine i nutritativna svojstva imaće primjenu u oplemenjivačkim programima za stvaranje novih sorti sa poboljšanim kvalitetom stočne hrane.

Ključne riječi: smiljkita, morfološke karakteristike, agronomska svojstva, korelativne veze.

Primljeno: 14. novembra 2023.
Odobreno: 6. juna 2024.

*Autor za kontakt: e-mail: vojo.radic@agro.unibl.org

Journal of Agricultural Sciences (Belgrade)
Vol. 69, No. 2, 2024
Pages 207-224

https://doi.org/10.2298/JAS2402207J
UDC: 633.18(669)
633.18:575.22
Original scientific paper

ANALYSIS OF GENOTYPIC AND PHENOTYPIC CORRELATIONS AND PATH COEFFICIENTS IN 40 GENOTYPES OF RAIN-FED UPLAND RICE (*ORYZA SATIVA* L.) IN OYO AND OGUN STATES REGIONS OF NIGERIA

Dans K. Johnson[1], Emmanuel O. Idehen[1,2] and Isaac O. Abegunde[1*]

[1]Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[2]Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

Abstract: Sustainable rice production in upland habitats depends on achieving higher yields. This study employs correlation and path coefficient analyses to identify essential trait criteria for enhancing rice yield in upland genotypes. The study included two growing seasons using 40 genotypes. Genotypic correlation analysis reveals a robust positive correlation of effective tillering with panicle number and yield. Notably, it shows significant negative correlations with 1000-grain weight and leaf width across diverse locations and cropping seasons. Additionally, the phenotypic estimates underscore a substantial positive correlation between yield and panicle number. Furthermore, the path analysis reveals that panicle number maintains a significantly positive association with yield at the 5% level of significance. Moreover, the analysis of the direct and indirect genotypic effects underscores the significance of culm number, effective tillering, and panicle number, all of which show remarkable and positive correlations with yield, achieving statistical significance at both the 5% and 1% levels. To enhance rice grain yield, a genotype must have an elevated count of pivotal traits per plant, including heightened panicle number, increased panicle length, greater culm number, elongated culm length, a greater number of effective tillers, early flowering initiation, expedited maturation, and augmented leaf length. These characteristics are pivotal determinants contributing significantly to the overall grain yield in rice cultivation and they are instrumental for sustainable rice improvement in the agro-ecology.

Key words: genetic diversity, rice improvement, yield, yield-related traits, genotype by environment interactions.

[*]Corresponding author: e-mail: ioabegunde@gmail.com

Introduction

Rice, one of the oldest and most important sources of energy for the human population, can be traced back to two species, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice), whereby the species name clearly reveals that rice originates from Africa and Asia. Rice plays a role as a cereal crop worldwide, as several studies have shown (Cheng et al., 2020). According to Nwanze et al. (2006), twenty million farmers around the world are involved in rice cultivation. It is one of the staple foods consumed globally in various forms (Kumar et al., 2020). More than half of the population relies on rice for their daily caloric intake, while approximately one billion households worldwide depend on its cultivation, processing, distribution, and marketing for their livelihoods (Asante et al., 2019). In Nigeria, rice production falls short of meeting consumption demands (Graham Acquaah et al., 2018), which leads to imports and higher prices that disadvantaged individuals find difficult to afford. Chen et al. (2020) have reported an increasing demand for high quality rice in China, potentially impacting the availability of rice grains in countries such as Nigeria that rely on imports, from other countries. Globally, upland rice accounts for 11% of rice grain production and is cultivated across 14 million hectares (Sohrabi et al., 2012). Although the overall percentage of rice production may not appear significant, upland rice is of great importance in tropical areas (Sohrabi et al., 2012). Comparing upland rice to wetland rice, the yield of upland rice is significantly lower. The average yield of wetland rice is around 3.30 tons per hectare, with a potential of 10 tons per hectare under ideal conditions. In contrast, upland rice yields range from 0.46 to 1.50 tons per hectare. To achieve self-sufficiency in rice production and meet the growing demand in regions with low rice yields, there is an urgent need to develop high-yielding upland rice varieties (Mulugeta et al., 2012). Although Nigeria has the potential for large-scale production, the lack of adaptable varieties has been one of its major setbacks. The approaches to generating adaptable varieties were based on the availability of desirable genetic variability for unique and important traits (Sumanth et al., 2017). Upland rice cultivation has gained popularity due to increased genetic variability caused by current high-yielding varieties, scarcity of irrigation water, and the breakdown of resistance genes to emerging pathogens due to intensive cultivation. In recent decades, the increase in global rice production has mainly benefited irrigated high-yielding varieties due to research breakthroughs and technology transfer. Upland rice research has been extremely limited, with most findings remaining unpublished in the study area. Consequently, these successes have had a negligible effect on upland rice production.

The main objective of rice breeders is to strive for higher yields either in both irrigated and non-irrigated environments. This involves trying to improve adaptable varieties through structured selection from breeding diverse parents and identifying

superior genotypes with desirable traits. Yield is a complex quantitative character that cannot be based on a single phenomenon. Other yield-contributing traits include plant height, leaf length, number of panicles per plant, number of grains per panicle, 1000-grain weight, days to flowering and days to maturity. The effective selection of superior genotypes for yield will associate yield with known attributing traits and consider these during the process (Neethu-Francis et al., 2018). The association among grain yields and yield component variables has been studied widely at the phenotypic level. Grain yield seems to have a significant correlation coefficient also with the number of filled grains per panicle, grain weight, panicle length, and grain count per panicle (Idris et al., 2012). Grain yield and number of grains per panicle, days to maturity, productive tillers, and days to flowering are clearly positively correlated (Sadeghi, 2011). Ullah et al. (2011) observed that grain yield and panicle length as well as the number of grains per panicle were positively and significantly related to each other. Hairmansis et al. (2010) discovered a relationship between grain yield and the number of filled grains per panicle, the number of spikelets per panicle, and spikelet fertility. Grain yield is considered as the most important characteristic, with high yield being the main goal of breeding in all crops. However, direct selection for yield is not very satisfactory as heritability is low. Several studies suggest using secondary traits, which include phenological, morphological, and physiological traits, as indirect selection criteria for higher yields. For instance, while correlation coefficients are important in determining what each secondary trait contributes to grain yield, they alone are not sufficient to determine whether the traits influence grain yield directly or indirectly (Nandan et al., 2010). The correlation coefficient can be divided into smaller components because one predictor variable has a direct effect on its response variable and because another predictor variable has a direct effect on the response variable through another predictor variable through path analysis, which breaks down the correlation coefficient into parts. Plant breeders use path analysis to find out which traits can be used as selection criteria to enhance crop yield (Sürek and Beser, 2003). Zou et al. (2005) have observed that spikelet fertility seems to be essential for grain yield under water deficit or drought-stress with a direct effect of P=0.60, whereas the number of spikelets per panicle makes the largest contribution to yield under well-watered conditions (P=0.41). In another study, Babu et al. (2012) found that the panicle length and the number of productive tillers on each plant had the most direct effect on yield through path coefficient analysis. Seyoum et al. (2012) found that the number of productive tillers, panicle weight, and spikelet fertility had positive and direct effects on grain yield in most of the studies analysed.

The aim of this study was to create several trait selection criteria for rice yield enhancement by using correlation and path coefficient analyses to find characteristics that have a direct effect on grain yield improvement in upland rice genotypes.

Material and Methods

The study was conducted in the 2019 and 2020 cropping seasons at two locations, the National Centre for Genetic Resources and Biotechnology (NACGRAB) in Ibadan, Oyo State and the Federal University of Agriculture Abeokuta (FUNAAB), Research and Experimental Farm in Ogun State using forty rice genotypes obtained from the Gene bank. Observations on culm length (cm), culm number, effective tillering, panicle length (cm), 1000-grain weight (g), plant height (cm), days to flowering, days to maturity, leaf length (cm), leaf width (mm), panicle number, grain length (mm), grain width (mm) and yield (kg plot $^{-1}$) were obtained and recorded.

The data obtained from the genotypes were subjected to correlation and path coefficient analyses. Estimates of genotypic and phenotypic correlations among yield and yield-related characters of the 40 genotypes of rice were conducted based on the environment using SAS version 9.4 (SAS Institute, 2000). The genotypic and phenotypic coefficients were partitioned to assess the direct and indirect effect of the yield-related trait on grain yield using path analysis in SAS software version 9.4.

Results and Discussion

The correlation coefficients for 14 quantitative traits among 40 rice genotypes across two locations and in two cropping seasons are presented in Table 1, differentiating between genotypic (upper diagonal) and phenotypic (lower diagonal) correlations. The genotypic correlation analysis reveals remarkable results. Culm length showed positive and significant correlations with panicle length, plant height, and leaf length. Similarly, culm number demonstrated positive and significant correlations with effective tillering and yield, while it exhibited a significant negative correlation with leaf width. Effective tillering displayed highly significant positive correlations with panicle number and yield, accompanied by significant negative correlations with 1000-grain weight and leaf width. Additionally, panicle length revealed highly significant positive correlations with plant height, leaf length, and grain length. Notably, 1000-grain weight exhibited highly significant negative correlations with panicle number and yield. These correlation analyses provide valuable insights into the relationships between these quantitative traits in rice genotypes under different environmental conditions and cropping seasons. The results highlight the significance of these traits for the expression of rice plant characteristics and ultimately for yield potential.

Plant height exhibited a remarkably strong and positive correlation with leaf length. Furthermore, days to flowering demonstrated a significant and positive correlation with days to maturity, while at the same time revealing a notable negative correlation with yield. Days to maturity exhibited a noteworthy negative

correlation with yield. The panicle number displayed a significant and positive correlation with yield. The phenotypic correlation analysis, as depicted in Table 1, unveiled a robust and positive association between yield and panicle number. In addition, grain width demonstrated a positive and significant correlation with grain length, while grain length exhibited a negative and significant correlation with effective tillering, but simultaneously displayed a positive and significant relationship with 1000-grain weight. Leaf length was positively and significantly correlated with culm length, effective tillering, and panicle length. Conversely, leaf width exhibited a negative and significant correlation with culm number. Days to maturity displayed a highly significant and positive correlation with days to flowering. Additionally, plant height showed a highly significant positive correlation with culm length, while panicle length also exhibited a highly significant positive correlation with culm length. Lastly, effective tillering displayed a highly significant and positive correlation with culm number.

Table 1. Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients of 14 quantitative characters in 40 rice genotypes at two locations in two cropping seasons.

Traits	CULL	CULN	EFFT	PAN	1000GRWT	PHT	DFLWR	DMATR	LFLN	LFWD	PANN	GRNL	GRNW	YLD
CULL		0.13	0.12	0.36*	-0.10	0.71**	-0.14	-0.11	0.59**	0.30	-0.02	-0.29	0.02	0.12
CULN	0.10		0.99**	-0.01	-0.29	-0.19	-0.11	-0.08	0.29	-0.56**	0.32*	-0.27	-0.13	0.34*
EFFT	0.11	0.89**		-0.02	-0.34*	-0.13	-0.05	-0.01	0.31	-0.52**	0.35*	-0.33*	-0.22	0.37*
PANL	0.23*	-0.09	-0.05		-0.14	0.63**	0.03	0.10	0.42*	0.00	-0.01	0.32*	-0.16	0.00
1000GRWT	-0.07	-0.16	-0.12	-0.08		0.09	-0.15	-0.13	-0.03	0.35	-0.38*	0.22	0.36	-0.36*
PHT	0.20*	-0.06	-0.06	0.10	-0.02		-0.09	-0.06	0.48*	0.37	-0.20	-0.13	-0.14	0.02
DFLWR	-0.12	-0.05	-0.04	-0.02	0.00	-0.01		1.00**	-0.22	-0.08	-0.28	-0.31	-0.1	-0.37*
DMATR	-0.09	-0.04	-0.01	-0.01	-0.02	0.00	0.88**		-0.10	-0.04	-0.32	-0.30	-0.16	-0.39*
LFLN	0.43**	0.21	0.24*	0.23*	-0.09	0.09	-0.16	-0.09		0.19	0.09	-0.13	-0.24	0.30
LFWD	0.10	-0.15*	-0.13	-0.01	0.07	0.05	-0.04	-0.08	0.13		-0.20	-0.11	-0.01	-0.01
PANN	-0.05	0.20	0.20	-0.06	-0.19	-0.04	-0.09	-0.11	0.05	-0.08		-0.13	0.06	0.87**
GRNL	-0.21	-0.13	-0.19*	0.14	0.17*	-0.04	-0.08	-0.08	-0.13	-0.04	-0.05		0.34	-0.24
GRNW	0.00	-0.01	-0.04	-0.09	0.20	-0.02	-0.07	-0.09	-0.08	-0.02	-0.01	0.18*		-0.09
YLD	0.07	0.21	0.21	0.03	-0.13*	0.01	-0.26*	-0.23*	0.14	-0.03	0.64**	-0.12	-0.04	

* and ** indicate significance at the 5% and 1% levels, respectively. CULL – culm length (cm), CULN – culm number, EFFT – effective tillering, PANL – panicle length (cm), 1000GRWT – 1000-grain weight (g), PHT – plant height (cm), FLWR – days to flowering, MATR – days to maturity, LFLN – leaf length (cm), LFWD – leaf width (mm), PANN – panicle number, GRNL – grain length (mm), GRNW – grain width (mm) and YLD – yield (kg/plot).

Table 2 shows the correlation coefficients for both genotypic and phenotypic traits for a dataset encompassing 40 different rice genotypes cultivated in Abeokuta during 2019. In the genotypic correlation analysis, culm length was found to have a significant positive correlation with panicle length, plant height, and leaf length.

Conversely, culm length displayed a noteworthy negative correlation with grain length. Additionally, culm number exhibited a positive and significant correlation with effective tillering, but a negative and significant correlation with 1000-grain weight. Notably, effective tillering displayed a highly significant negative correlation with 1000-grain weight. Furthermore, panicle length showed a strong positive correlation with plant height (0.46) and leaf length. It was evident that 1000-grain weight was negatively correlated with panicle number, while it showed a significant positive correlation with grain width and yield. Additionally, plant height exhibited a highly significant positive correlation with leaf length and leaf width. However, it was significantly negatively correlated with grain length. Furthermore, a significant and positive correlation was observed between days to flowering and days to maturity. Conversely, days to maturity displayed a negatively significant correlation with yield.

Table 2. Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients among yield and yield-related characters in Abeokuta in 2019.

Traits	CULL	CULN	EFFT	PANL	1000GRWT	PHT	DFLWR	DMATR	LFLN	LFWD	PANN	GRNL	GRNW	YLD
CULL		0.04	0.06	0.33*	-0.03	0.85**	-0.22	-0.27	0.60**	0.40*	-0.17	-0.37*	-0.20	0.09
CULN	0.03		0.99**	-0.06	-0.37*	0.00	-0.01	-0.05	0.20	-0.28	0.18	-0.19	-0.24	0.30
EFFT	0.05	0.98**		-0.02	-0.36*	0.01	0.00	-0.03	0.26	-0.23	0.16	-0.24	-0.29	0.29
PANL	0.32*	-0.06	-0.02		-0.03	0.46**	-0.12	-0.13	0.58**	0.14	-0.12	0.07	-0.21	-0.09
GRWT	-0.04	-0.39*	-0.39*	-0.03		-0.04	-0.13	-0.13	0.05	0.18	-0.32*	0.23	0.34*	-0.26
PHT	0.84**	0.00	0.01	0.44**	-0.04		-0.25	-0.24	0.66**	0.45**	-0.14	-0.33*	-0.28	0.10
FLWR	-0.22	0.01	0.02	-0.11	-0.12	-0.25		1.00**	-0.26	-0.06	-0.1	-0.16	-0.09	-0.30
MATR	-0.25	-0.04	-0.02	-0.11	-0.11	-0.23	0.95**		-0.26	-0.05	-0.17	-0.18	-0.08	-0.39*
LFLN	0.56**	0.18	0.24	0.52**	0.03	0.62**	-0.25	-0.25		0.41**	0.00	-0.12	-0.16	0.24
LFWD	0.38*	-0.26	-0.2	0.13	0.17	0.43	-0.06	-0.05	0.36*		-0.29	-0.13	-0.13	-0.23
PANN	-0.18	0.16	0.14	-0.12	-0.34	-0.14	-0.09	-0.15	0.00	-0.27		-0.05	-0.12	0.75**
GRNL	-0.37*	-0.21	-0.26	0.05	0.20	-0.32*	-0.14	-0.15	-0.12	-0.11	-0.07		0.60**	-0.14
GRNW	-0.18	-0.21	-0.26	-0.18	0.30	-0.25	-0.07	-0.08	-0.16	-0.12	-0.11	0.50**		0.01
YLD	0.07	0.28	0.27	-0.10	-0.28	0.10	-0.28	-0.34*	0.22	-0.20	0.72**	-0.15	-0.01	

* and ** indicate significance at the 5% and 1% levels, respectively. CULL – culm length (cm), CULN – culm number, EFFT – effective tillering, PANL – panicle length (cm), 1000GRWT – 1000-grain weight (g), PHT – plant height (cm), FLWR – days to flowering, MATR – days to maturity, LFLN – leaf length (cm), LFWD – leaf width (mm), PANN – panicle number, GRNL – grain length (mm), GRNW – grain width (mm) and YLD – yield (kg/plot).

In the context of the phenotypic correlation analysis (as shown in Table 2), several significant associations were observed. Specifically, yield exhibited a notable negative and significant correlation with days to maturity, while it displayed a positive and significant correlation with panicle number due to the genetic nature of the materials under evaluation and some interactions with the environment. Furthermore, grain width displayed a significant and positive

correlation with grain length. In contrast, grain length exhibited a negative and significant correlation with culm length and plant height. Additionally, leaf width showed positive and significant correlations with culm length and leaf length. Leaf length was positively and significantly correlated with culm length, panicle length, and plant height. In 2019, there was a highly significant positive correlation between days to maturity and days to flowering in Abeokuta (as shown in Table 2). Plant height exhibited highly significant positive correlations with culm length and panicle length. Additionally, 1000-grain weight demonstrated highly significant negative correlations with culm number and effective tillering (-0.39 each). Effective tillering, on the other hand, exhibited a significant positive correlation with culm number. Furthermore, the correlation coefficients for 14 quantitative traits among 40 rice genotypes in Abeokuta in 2020 (as shown in Table 3) highlighted significant associations. Notably, culm length displayed positive and significant associations with panicle length, plant height, and leaf length, while negatively and significantly correlating with 1000-grain weight. Culm number exhibited positive and significant correlations with effective tillering and leaf length but was negatively and significantly correlated with panicle length.

Table 3. Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients among yield and yield-related characters in Abeokuta in 2020.

Traits	CULL	CULN	EFFT	PANL	1000GRWT	PHT	FLWR	MATR	LFLN	LFWD	PANN	GRNL	GRNW	YLD
CULL		0.12	0.19	0.40*	-0.33*	0.75**	-0.06	-0.03	0.68**	NA	-0.01	-0.29	0.19	-0.06
CULN	0.10		0.97**	-0.35*	-0.20	-0.04	-0.04	0.06	0.33*	NA	0.37*	-0.09	0.26	0.15
EFFT	0.17	0.94**		-0.37*	-0.31	0.02	0.01	0.12	0.35*	NA	0.33*	-0.25	0.12	0.17
PANL	0.30	-0.29	-0.29		-0.60**	0.51**	-0.1	-0.04	0.21	NA	0.33*	0.25	0.39*	0.31
GRWT	-0.23	-0.15	-0.12	-0.27		-0.24	0,00	-0.01	-0.30	NA	-0.42**	0.28	0.18	-0.39*
PHT	0.68**	-0.04	0.01	0.38*	-0.12		-0.17	-0.1	0.63**	NA	-0.04	-0.19	-0.31*	0.09
FLWR	-0.06	-0.03	0.02	-0.05	0.03	-0.14		1.00**	0.27	NA	-0.20	-0.16	-0.18	-0.29
MATR	-0.03	0.07	0.12	0.00	0.01	-0.08	0.95**		0.29	NA	-0.22	-0.21	-0.25	-0.22
LFLN	0.66**	0.31*	0.32*	0.15	-0.18	0.54**	0.23	0.25		NA	0.02	-0.21	-0.19	-0.10
LFWD	-0.04	-0.06	-0.04	-0.41**	-0.04	-0.02	-0.01	-0.09	-0.06		NA	NA	NA	NA
PANN	-0.02	0.28	0.25	0.15	-0.29	-0.06	-0.16	-0.17	0.02	0.03		-0.07	-0.13	0.81**
GRNL	-0.27	-0.13	-0.24	0.18	0.15	-0.20	-0.13	-0.12	-0.17	0.05	-0.04		0.04	-0.17
GRNW	0.12	0.14	0.07	0.07	0.13	-0.18	-0.11	-0.10	-0.07	0.11	-0.03	0.01		-0.23
YLD	-0.06	0.15	0.15	0.23	-0.28	0.08	-0.27	-0.20	-0.10	0.02	0.75**	-0.14	-0.10	

* and ** indicate significance at the 5% and 1% levels, respectively. CULN – culm number, EFFT – effective tillering, PANL – panicle length (cm), 1000GRWT – 1000-grain weight (g), PHT – plant height (cm), FLWR – days to flowering, MATR – days to maturity, LFLN – leaf length (cm), LFWD – leaf width (mm), PANN – panicle number, GRNL – grain length (mm), GRNW – grain width (mm) and YLD – yield (kg/plot).

In the correlation analysis, several associations were observed among different traits in rice plants. Panicle length showed a significant negative correlation with

1000-grain weight, but a positive correlation with plant height. Conversely, 1000-grain weight had a significant negative correlation with panicle number. Plant height had a significant positive correlation with leaf length, but a significant negative correlation with grain width. Days to flowering had a significant positive correlation with days to maturity, indicating a connection between these developmental stages. Panicle number showed a significant positive correlation with yield, highlighting its importance for grain production. Yield was positively associated with panicle number, indicating its significance as a yield determinant. Leaf width had a significant negative correlation with panicle length. Leaf length exhibited highly significant positive correlations with culm length, culm number, effective tillering, and plant height, showing its multifaceted relationship with various traits. Plant height had positive and significant correlations with culm length and panicle length, emphasising its influence on these traits. Effective tillering had a significant positive correlation with culm number, indicating its role in promoting culm development. In genotypic correlation analysis among 40 rice genotypes in Ibadan in 2019 (as detailed in Table 4), culm length was positively associated with panicle length, plant height, leaf length, and leaf width, but negatively associated with 1000-grain weight. Culm number had a positive correlation with effective tillering, but negative correlations with 1000-grain weight, leaf width, and grain width.

The correlations clearly show that effective tillering exhibited a favourable correlation with leaf length, while simultaneously displaying highly significant negative correlations with 1000-grain weight, grain length, and grain width. Moreover, panicle length emerges as a key factor, showing highly significant positive correlations with plant height and leaf length. In contrast, 1000-grain weight unveiled a complex relationship, showing highly significant negative correlation with panicle number and a positive correlation with grain width. The stature of the rice plants cannot be overlooked, as plant height exhibited highly significant positive correlations with both leaf length and leaf width. Days to flowering and days to maturity exhibited significant correlations, with the former displaying a positive correlation with the latter and a negative correlation with yield. Days to maturity, on the other hand, presented a significant negative correlation with yield. Notably, panicle number emerged as a vital factor, establishing a significantly positive correlation with yield.

The exploration of phenotypic correlations, as illustrated in Table 4, illuminates the relationship between yield and other traits. Yield, the ultimate measure of success, showed a significant positive association with panicle number but also showed a negative correlation with days to flowering. Leaf width stood out with its noteworthy relationships, displaying significantly positive correlations with culm length and plant height, while concurrently manifesting a significant negative correlation with culm number. Grain width showed a significant negative

correlation with effective tillering. Panicle number, a key determinant of yield, displayed a highly significant negative correlation with 1000-grain weight and leaf width. The towering stature of the rice plants, which is expressed by plant height, revealed significant positive correlations with both culm length and panicle length. Further investigation of 1000-grain weight unveiled its intricate associations, with highly significant negative correlations with culm number and effective tillering. Additionally, panicle length exhibited a significant positive correlation with culm length, emphasising the synergy between these traits. The importance of effective tillering was underscored by its significant positive correlation with culm number, highlighting its role in determining yield potential.

Table 4. Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients among yield and yield-related characters in Ibadan in 2019.

Traits	CULL	CULN	EFFT	PANL	GRWT	PHT	FLWR	MATR	LFLN	LFWD	PANN	GRNL	GRNW	YLD
CULL		0.07	0.07	0.42**	-0.04	0.85**	-0.25	-0.23	0.46**	0.36*	-0.12	-0.33*	0.07	0.13
CULN	0.06		0.99**	0.05	-0.33*	0.05	-0.04	-0.03	0.37*	-0.33*	0.17	-0.31	-0.34*	0.24
EFFT	0.05	0.98**		0.06	-0.31*	0.06	0.03	0.04	0.38*	-0.30	0.11	-0.32*	-0.41**	0.20
PANL	0.39*	0.03	0.04		0.00	0.45**	-0.20	-0.23	0.51**	0.19	-0.18	0.11	-0.23	-0.08
GRWT	-0.06	-0.35*	-0.33*	-0.01		0.00	-0.13	-0.14	-0.03	0.28	-0.37*	0.17	0.46**	-0.21
PHT	0.84*	0.06	0.06	0.42**	-0.01		-0.26	-0.25	0.44*	0.40*	-0.16	-0.28	-0.15	0.13
FLWR	-0.24	-0.02	0.04	-0.19	-0.11	-0.25		1.00**	-0.34*	-0.12	-0.07	-0.07	-0.03	-0.35*
MATR	-0.22	-0.01	0.05	-0.22	-0.12	-0.24	0.99**		-0.31	-0.12	-0.07	-0.08	0.00	-0.34*
LFLN	0.45**	0.35*	0.36*	0.46**	-0.04	0.43**	-0.33*	-0.30		0.31*	0.01	-0.07	-0.27	0.24
LFWD	0.36*	-0.31*	-0.29	0.18	0.28	0.38*	-0.12	-0.12	0.31		-0.35*	-0.18	-0.26	-0.23
PANN	-0.13	0.15	0.09	-0.18	-0.38*	-0.15	-0.06	-0.05	0.00	-0.32*		-0.16	0.08	0.85**
GRNL	-0.31	-0.28	-0.28	0.08	0.12	-0.23	-0.04	-0.05	-0.1	-0.15	-0.16		0.34*	-0.29
GRNW	0.00	-0.30	-0.34*	-0.21	0.27	-0.09	0.01	0.03	-0.22	-0.16	0.02	0.26		-0.14
YLD	0.10	0.21	0.17	-0.09	-0.23	0.13	-0.33*	-0.31	0.22	-0.22	0.80**	-0.27	-0.13	

* and ** indicate significance at the 5% and 1% levels, respectively. CULL – culm length (cm), CULN – culm number, EFFT – effective tillering, PANL – panicle length (cm), 1000GRWT – 1000-grain weight (g), PHT – plant height (cm), FLWR – days to flowering, MATR – days to maturity, LFLN – leaf length (cm), LFWD – leaf width (mm), PANN – panicle number, GRNL – grain length (mm) , GRNW – grain width (mm) and YLD – yield (kg/plot).

The results pertaining to the genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients of 14 quantitative characteristics of rice in the 2020 Ibadan study are meticulously laid out in Table 5. The genotypic analysis clearly shows that culm length showed a significantly positive association with panicle length and leaf length. In a parallel vein, culm number established a noteworthy positive correlation with effective tillering and yield, while concurrently displaying a substantial negative correlation with leaf width. The parameter of effective tillering emerged as a pivotal factor, unveiling highly significant negative correlations with 1000-grain weight and leaf width, along with a positive correlation with panicle number and yield. Meanwhile, panicle length

proved to be a key determinant and showed highly significant positive correlations with leaf length, leaf width, and grain length. The intricate web of correlations extends further, as 1000-grain weight established a significant negative correlation with days to flowering, days to maturity, panicle number, and yield. In contrast, 1000-grain weight demonstrated significant positive correlations with leaf length, leaf width, grain length, and grain width. It was observed that days to flowering exhibited a significant positive correlation with days to maturity, while simultaneously displaying a negative correlation with leaf width, grain width, and yield. Days to maturity, on the other hand, exhibited significant negative correlations with grain length, grain width, and yield. Leaf width, an often-overlooked trait, showed a significant negative correlation with panicle number and a substantial positive correlation with grain length. Panicle number, a prominent player in determining yield, established a significant positive correlation with yield, while simultaneously manifesting a substantial negative correlation with grain length. When analysing phenotypic direct and indirect effects, Figure 1 illuminates that four traits are statistically significant. Among them, grain weight, flowering, and maturity exhibited significant negative correlations with yield at the 5% level of significance, underscoring their influence on yield dynamics. In stark contrast, panicle number proved to be an outstanding influencing factor with a significantly positive correlation with yield at the remarkable 1% level of significance. The path analysis, as delineated in Figure 2, provides a deeper understanding of the genotypic direct and indirect determinants of yield in the 40 rice genotypes. Within this intricate interplay, four traits emerged as statistically significant. Notably, grain weight, flowering, and maturity were found to have significant negative correlations with yield at the 5% level of significance, casting a shadow on yield prospects. In a parallel vein, culm number, effective tillering, and panicle number were found to be the driving forces behind yield, as they showed significantly positive correlations with yield, distinguished at both the 5% and 1% levels of significance.

Such interrelationships can provide valuable insights into indirect selection strategies aimed at enhancing overall yield, as shown by Htwe et al. (2019). Notably, a robust and consistently positive genotypic and phenotypic correlation surfaced between yield per plant and panicle number per plant across all environments. This finding underscores the significance of panicle number as a major factor in increasing grain yield. It further highlights that direct selection for panicle number can be an effective strategy to increase rice yields. In the realm of trait evaluation, the reliability of inter-character association estimates depends on the presence of both significant genotypic and phenotypic correlations, as explained by Ogunbayo et al. (2014). The substantial and consistently positive genotypic and phenotypic correlations identified in various environments, including those between plant height and culm length, effective tillering and culm

number, days to flowering and days to maturity, leaf length and culm length, and leaf length and panicle length, underscore the central role that these traits play in relation to grain yield. This robust correlation framework is convincing evidence that these traits are of paramount importance in the context of grain yield enhancement.

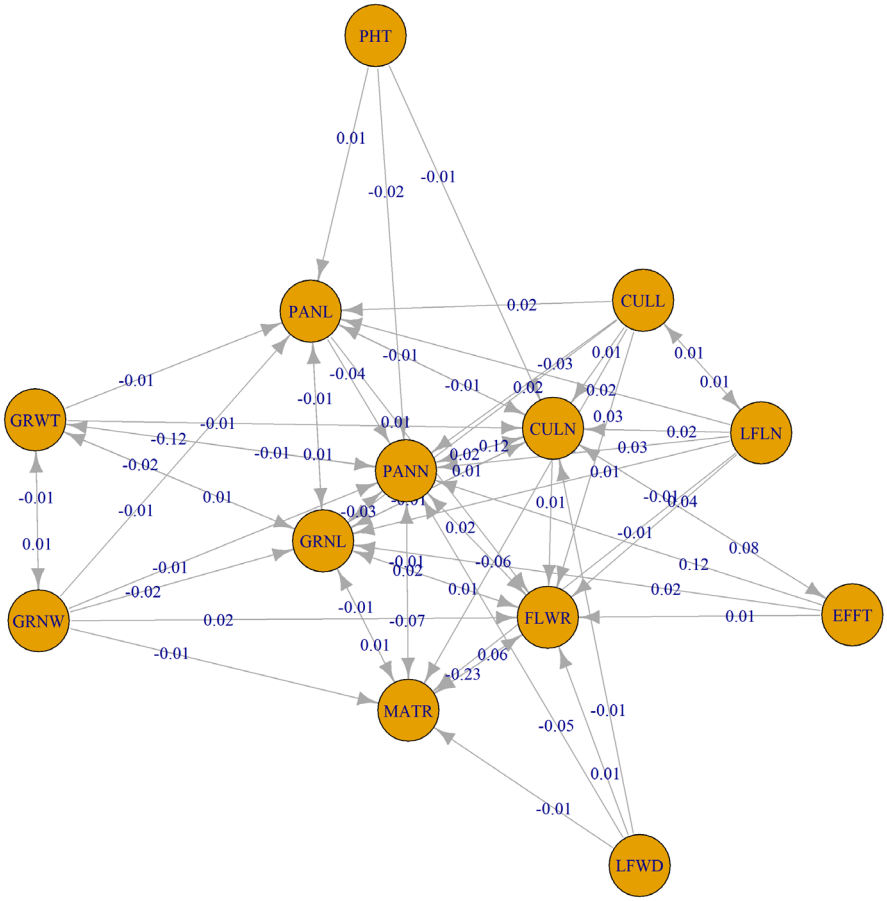

Figure 1. Phenotypic direct and indirect effects of characters on the yield of 40 rice genotypes for path analysis.

Significant at * P < 0.05; ** P < 0.01, Residual = 0.72, CULL – culm length (cm), CULN – culm number, EFFT – effective tillering, PANL – panicle length (cm), 1000GRWT – 1000-grain weight (g), PHT – plant height (cm), FLWR – days to flowering, MATR – days to maturity, LFLN – leaf length (cm), LFWD – leaf width (mm), PANN – panicle number, GRNL – grain length (mm), GRNW – grain width (mm).

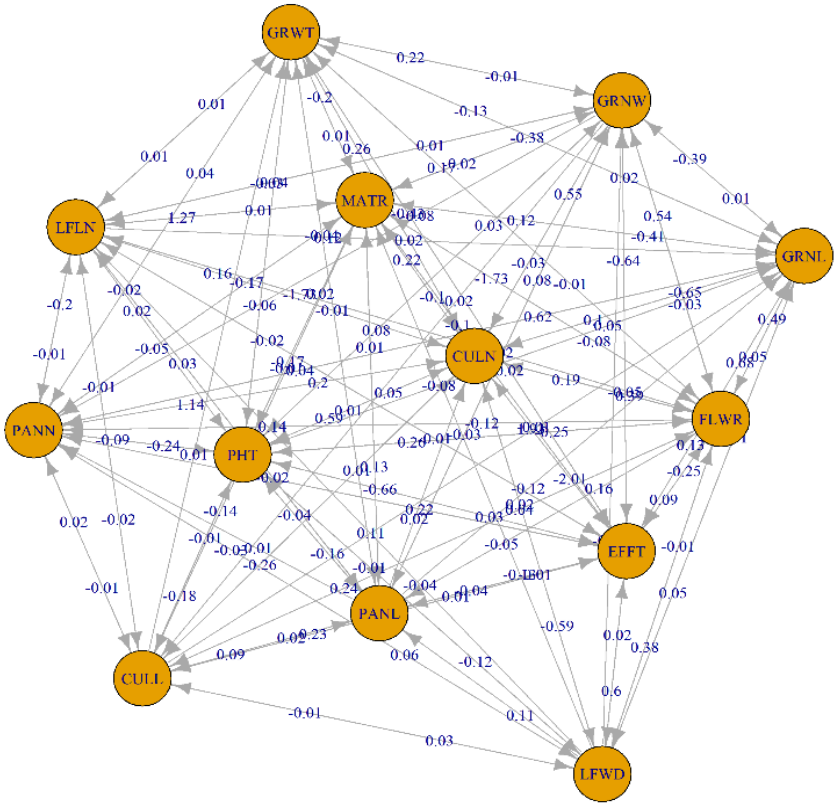

Figure 2. Genotypic direct and indirect effects of characters on the yield of 40 rice genotypes for path analysis.

Significant at * P < 0.05; ** P < 0.01, Residual = 0.44, CULL – culm length (cm), CULN – culm number, EFFT – effective tillering, PANL – panicle length (cm), 1000GRWT – 1000-grain weight (g), PHT – plant height (cm), FLWR – days to flowering, MATR – days to maturity, LFLN – leaf length (cm), LFWD – leaf width (mm), PANN – panicle number, GRNL – grain length (mm), GRNW – grain width (mm).

This also underscores the effectiveness of focusing selection efforts on these specific traits to ensure improved grain yield in rice. This observation aligns with the findings of Htwe et al. (2019), who also documented substantial positive genotypic and phenotypic correlations between yield per plant and key indicators such as the effective tillering, panicle/straw ratio, harvest index, filled grain percentage, and the number of spikelets per panicle. These results are consistent with the research of Ogunbayo et al. (2014), who revealed a significant positive

association between grain yield and the number of panicles per plant, and Ramakrishman et al. (2006), who reported a similar correlation for the number of spikelets per panicle. Conversely, yield per plant exhibited a significant negative correlation with 1000-grain weight, days to flowering, and days to maturity. Thus, enhancing grain yield per plant could be achieved by selecting genotypes with early flowering and maturation. These findings are consistent with those of Htwe et al. (2019), who also identified negative correlations between grain yield and days to flowering, panicle length, and 1000-grain weight. Consequently, based on the insights derived from correlation studies, it can be inferred that genotype should possess a higher number of panicles per plant, a greater panicle length per plant, an increased culm number per plant, a longer culm length per plant, a higher number of effective tillers, early flowering, and maturation, and a longer leaf length in order to increase the grain yield of rice.

Table 5. Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients among yield and yield-related characters in Ibadan in 2020.

Traits	CULL	CULN	EFFT	PANL	1000GRWT	PHT	FLWR	MATR	LFLN	LFWD	PANN	GRNL	GRNW	YLD
CULL		0.17	0.04	0.34*	0.17	NA	0.11	0.2	0.54**	-0.06	0.04	-0.1	0.06	0.07
CULN	0.13		0.94**	-0.1	-0.19	NA	-0.13	-0.24	-0.1	-0.60**	0.31	0	0.11	0.33*
EFFT	0.05	0.87**		0.02	-0.43**	NA	-0.19	-0.2	0.05	-0.64**	0.43**	-0.19	0.01	0.39*
PANL	0.22	-0.19	-0.08		0.01	NA	0.12	0.21	0.85**	0.45**	0.23	0.62**	-0.22	0.17
GRWT	0.12	-0.12	-0.23	-0.07		NA	-0.40*	-0.52**	0.39**	0.47**	-0.43**	0.96**	0.38*	-0.34*
PHT	-0.02	-0.17	-0.27	0.03	-0.02		NA	NA	NA	NA	NA	NA	NA	NA
FLWR	0.08	-0.13	-0.17	0.17	0.06	0.03		0.98**	-0.25	-0.34*	0.1	0.3	-0.77**	-0.49**
MATR	0.13	-0.18	-0.16	0.15	-0.05	0.06	0.80**		-0.24	-0.23	-0.13	-0.87**	-0.87**	-0.48**
LFLN	0.39*	-0.05	0.06	0.29	0.09	-0.3	-0.16	-0.09		0.29	0.31	-0.11	0.04	0.23
LFWD	-0.01	-0.48**	-0.51**	0.19	0.33*	0.32*	-0.18	-0.1	0.18		-0.57**	0.56**	0.28	-0.04
PANN	0.02	0.24	0.34*	0.09	-0.21	-0.16	0.08	-0.06	0.22	-0.39*		-0.48**	0.03	0.51**
GRNL	-0.13	0.03	-0.11	0.16	0.35*	0.15	0.06	-0.2	-0.13	0.17	-0.23		0.51**	-0.35*
GRNW	0.04	0.12	0.03	-0.11	0.32*	0	-0.38*	-0.52**	0	0.07	0.06	0.31		-0.02
YLD	0.08	0.21	0.29	0.16	-0.17	-0.17	-0.24	-0.26	0.17	-0.08	0.53**	-0.18	-0.06	

* and ** indicate significance at the 5% and 1% levels, respectively. CULL – culm length (cm), CULN – culm number, EFFT – effective tillering, PANL – panicle length (cm), 1000GRWT – 1000-grain weight (g), PHT – plant height (cm), FLWR – days to flowering, MATR – days to maturity, LFLN – leaf length (cm), LFWD – leaf width (mm), PANN – panicle number, GRNL – grain length (mm), GRNW – grain width (mm) and YLD – yield (kg/plot).

The estimation of genotypic and phenotypic correlation coefficients is very useful in plant breeding, facilitating selection based on phenotypic performance. In the context of Abeokuta in 2019, the computed genotypic and phenotypic correlation coefficients for the number of days to maturity and yield, as well as for panicle number and yield were notably significant. These results imply that the selection of early maturing genotypes and those with a higher number of panicles can effectively contribute to an increased yield. Conversely, in Abeokuta 2020, the negative

correlation observed between grain width and yield suggests an inverse relationship. To enhance yield, grains with a reduced width would therefore have to be selected.

The noteworthy negative correlation between the number of days to flowering and maturity and yield observed in Ibadan during 2019 implies that enhancing the genotypes requires the selection of early flowering and early maturing plants. Conversely, the significant correlations between culm number, effective tillering, and panicle number with yield in Ibadan in 2020 suggest that improving these traits will ultimately lead to increased yield. However, the negative correlations observed between grain width, the number of days to flowering and maturity with yield suggest that reducing these traits would result in a simultaneous increase in yield. It is worth noting that the number of genetic components influencing the direction of association between traits tends to vary with the environment, as highlighted by Obilana and Fakorede (1986) and Bänziger et al. (2006). As Shukla et al. (2004) point out, a comprehensive understanding of the genetic parameters in different environments is therefore essential to enable effective selection and progress in breeding programmes.

Path analysis, a valuable tool, allows the separation of direct effects and corresponding indirect effects through other attributes, providing a more comprehensive explanation of cause and effect (Sadeghi, 2011). In the context of path analysis, grain yield per plant serves as the dependent variable. The study assessed the direct and indirect phenotypic effects of various characters. Panicle number exhibited the most substantial direct phenotypic effect on grain yield. Furthermore, panicle number displayed a highly significant positive correlation with grain yield per plant. A separate study conducted by Htwe et al. (2019) also reported that spikelets per panicle, effective tillers per plant, and filled grain percentage had direct effects on yield, with these characters demonstrating positive and highly significant correlations with yield per plant.

The direct and indirect contributions of the traits to the genotypic correlation revealed a substantial and positive direct effect of panicle number, culm number, and effective tillering on the rice grain yield of the 40 genotypes. These traits should be adequately considered when selecting for yield among the cultivars. Neethu-Francis et al. (2018) also reported a significantly positive direct effect of the number of panicles per plant, the number of grains per panicle, and 1000-grain weight on the yield of rice cultivars.

The negative direct effect of these traits on yield has also been reported by Neethu-Francis et al. (2018) and Devendra et al. (2016), aligning with the findings of this study. Other traits showed a negative indirect effect on grain yield through grain weight, days to flowering, and days to maturity, and a moderately positive indirect influence on grain yield through culm number and effective tillering. Htwe et al. (2019) also reported that the number of spikelets per panicle had a positive direct effect on yield per plant of Myanmar local rice. Additionally, panicle/straw

weight ratio and panicle length were found to have a high indirect effect via effective tillering on the yield of Myanmar local rice (Htwe et al., 2019).

The fact that five traits (effective tillering, panicle length, days to maturity, panicle number, and grain width) exhibited a positive direct effect on grain yield at the genotypic level suggests that any of these traits can be used in a rice improvement programme under the conditions of the study. However, the positive indirect effect of culm number, grain weight, plant height, days to flowering, leaf length, leaf width, and grain length can offset the negative direct effect on grain yield. Therefore, it is advisable to prioritise all the traits studied in rice improvement. Akinyele and Osekita (2006) noted that high indirect effects of traits can counterbalance low direct effects of traits on crop yield.

The residual effect of 0.72 at the phenotypic level indicates that the selected characters contributed to 28% of the variability. At the genotypic level, the residual effect was 0.44, suggesting that the characters studied in this research contributed to 56% of the variability. According to Htwe et al. (2019), the residual effect typically determines how effectively the causal factors account for the variability of the dependent factors, such as the standard evaluation score.

Conclusion

Panicle number directly impacts grain yield, with more panicles leading to higher yields. Other factors such as panicle length, culm number, effective tillering, and days to maturity also influence grain yield. In all environments, positive correlations were found between yield per plant and panicle number per plant, establishing the importance of panicle number in increasing grain yield. To improve rice grain yield, varieties should possess traits such as increased panicle and culm numbers, longer panicles and culms, more effective tillering, early flowering and maturation, and greater leaf length per plant. These traits significantly correlate with yield. In Abeokuta in 2019 and 2020, traits such as panicle number, panicle length, effective tillering, culm length, plant height, leaf length, days to flowering, days to maturity, grain length, and grain width were found to have correlated with grain yield.

Culm length, culm number, effective tillering, plant height, days to flowering, panicle number and days to maturity showed correlated responses to grain yield in Ibadan in 2019. In 2020, traits such as culm number, effective tillering, grain length, 1000-grain weight, panicle number, days to flowering and days to maturity were correlated with grain yield in Ibadan. Developing improved rice genotypes that are adaptable to diverse environmental conditions is advisable, as the traits in the study area were mainly influenced by genetics and may be subjective in other locations under similar environmental conditions. It is therefore recommended that environmental conditions be considered as a factor in the quest to genetically improve rice and ensure sustainable production in the agro-ecology of Nigeria.

References

Akinyele, B.O., & Osekita, O.S. (2006). Correlation and path coefficient analyses of seed yield attributes in okra (*Abelmoschus esculentus* L Moench). *Africa Journal of Biotechnology, 5* (14), 1330-1336.

Asante, M.D., Adjah, K.L., & Annan-Affu, E. (2019). Assessment of genetic diversity for grain yield and yield component traits in some genotypes of rice (*Oryza Sativa* L.). *Journal of Crop Science and Biotechnology*, *22* (2), 123-130.

Babu, V.R., Shreya, K., Dangi, K.S., Usharani, G., & Shankar, A.S. (2012). Correlation and path analysis studies in popular rice hybrids of India. *International Journal of Scientific and Research Publications*, *2*, 1-5

Bänziger, M., Setimela, P.S., Hodson, D., & Vivek, B. (2006). Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. *Agricultural Water Management, 80* (1-3), 212-224. https://doi.org/10.1016/j.agwat.2005.07.014.

Chen, S., Liu, S., Yin, M., Zheng, X., Chu, G., Xu, C., Wang, D., & Zhang, X. (2020). Seasonal changes in crop growth and grain yield of different japonica rice cultivars in southeast China. *Agronomy Journal*, *112* (1), 1-13.

Cheng, F., Quan, X., Zhengjin, X., & Wenfu, C. (2020). Effect of Rice Breeding Process on Improvement of Yield and Quality in China. *Rice Science*, *27* (5), 363-367.

Devendra, S.J., Roshan, P., Prakash, A.A., Grace, M.M., & Sara, N. (2016). Correlation and path coefficient studies for grain yield and other yield attributes on aromatic short grain rice (*Oryza sativa* L.) genotypes. *International Journal of Agriculture of Science, 8* (51), 2318-2320.

Graham-Acquaah, S., Saito, K., Traore, K., Dieng, I., Alognon, A., Bah, S., Sow, A., & Manful, J.T. (2018). Variations in agronomic and grain quality traits of rice grown under irrigated lowland conditions in West Africa. *Food Science and Nutrition*, *6*, 970-982.

Hairmansis, A., Kustianto, B., & Supartopo, S. (2010). Correlation analysis of agronomic characters and grain yield of rice for tidal swamp areas. *Indonesian Journal of Agricultural, Science, 11*, 11-15.

Htwe, N.M., Phyu, S.L., & Thu, C.N. (2019). Assessment of Genetic Variability and Character Association of Myanmar Local Rice (*Oryza sativa* L.) Germplasm. *Journal of Experimental Agriculture International, 40* (3), 1-10

Idris, A.E., Justin, F.J., Dagash, Y.M.I., & Abuali, A.I. (2012). Genetic Variability and Inter Relationship between Yield and Yield Components in some Rice Genotypes. *American Journal of Experimental Agriculture, 2* (2), 233-239. http://dx.doi.org/10.9734/AJEA/2012/961

Kumar, A., Raman, A., Yadav, S., Verulkar, S.B., Mandal, N.P., Singh, O.N., Swain, P., Ram, T., Badri, J., Dwivedi, J.L., Das, S.P., Singh, S.K., Singh, S.P., Kumar, S., Jain, A., Chandrababu, R., Robin, S., Shashidhar, H.E., Hittalmani, S., Satyanarayana, P., Venkateshwarlu, C., Ramayya, J., Naik, J., Nayak, S., Dar, M.H., Hossain, S.M, Henry, A., & Piepho, H.P. (2020). Genetic gain for rice yield in rainfed environments in India. *Field Crops Research, 260*, 107977. doi: 10.1016/j.fcr.2020.107977

Mulugeta, S., Sentayehu, A., & Kassahun, B. (2012). Genetic variability, heritability, correlation coefficient and path analysis for yield and yield related traits in upland rice (*Oryza sativa* L.). *Journal of Plant Sciences*, *7* (1), 13-22.

Nandan, R., Sweta, & Singh, S.K. (2010). Character association and path analysis in rice (*Oryza sativa* L.) genotypes. *World Journal of Agricultural Sciences*, *6*, 201-206.

Neethu-Francis, D., Packiaraj, S., Geethanjali, & Hemaprabha, K. (2018). Correlation and Path Coefficient Analysis for Yield Contributing Characters in Rice (*Oryza sativa* L.) Cultivars. *International Journal of Current Microbiology and Applied Sciences, 7* (6), 2292-2296. doi: https://doi.org/10.20546/ijcmas.2018.706.274

Nwanze, K.F., Mohapatra, S., Kormawa, P., Keya, S., & Bruce-Oliver, S. (2006). Rice development in subSaharan Africa. *Journal of the Science of Food and Agriculture*, *86*, 675-677.

Obilana, A.T., & Fakorede M.A.B. (1986). Heritability: A Treatise. *Samaru Journal of Agricultural Research, 1*, 72-82.

Ogunbayo, S.A., Sié M., Ojo, D.K., Sanni, K.A., Akinwale, M.G., Toulou, B., Shittu, A., Idehen, E.O., Popoola, A.R., Daniel, I.O., & Gregorio, G.B. (2014). Genetic variation and heritability of yield and related traits in promising ricegenotypes (Oryza sativa L.*). Journal of Plant Breeding and Crop Science*, *6* (11), 153-159. DOI: 10.5897/JPBCS2014.0457

Ramakrishman, S.H., Amandakumar, C.R., Saravanan, S., & Malini, N. (2006). Association Analysis of some yield traits in rice (*Oryza sativa* L.). *Journal of Applied Sciences Research*, *2* (7), 402-404.

Sadeghi, S.M. (2011). Heritability, phenotypic correlation, and path coefficient studies for some agronomic characters in landrace rice varieties. *World Applied Sciences Journal*, *13*, 1229-1233.

SAS Institute (2000). SAS Linear Model: A Guide to ANOVA and GLM Procedures. SAS Inst. Inc., Cary, NC, USA.

Seyoum, M., Alamerew, S., & Bantte, K. (2012). Genetic variability, heritability, correlation coefficient and path analysis for yield and yield related traits in upland rice (*Oryza sativa* L.). *Journal of Plant Sciences*, *7*, 13-22.

Shukla, S., Bhargava, A., Chatterjee, A., & Singh, S.P. (2004). Estimates of genetic parameters to determine variability for foliage yield and its different quantitative and qualitative traits in vegetable amaranth (*A. tricolor*). *Journal of Genetics and Breeding, 58*, 169-176.

Sohrabi, M., Rafii, M.Y., Hanafi, M.M., Akmar, A.S.N., & Latif, M.A. (2012). Genetic diversity of upland rice germplasm in Malaysia based on quantitative traits. *The Scientific World Journal*, Volume 2012, Article ID 416291. doi:10.1100/2012/416291

Sumanth, V., Suresh, B.G., Ram, B.J., & Srujana, G. (2017). Estimation of genetic variability, heritability, and genetic advance for grain yield components in rice (*Oryza sativa* L.). *Journal of Pharmacognosy and Phytochemistry, 6*, 1437-1439.

Sürek, H., & Beser, N. (2003). Correlation and path coefficient analysis for some yield related traits in rice (*Oryza sativa* L.) under Thrace. *Turkish Journal of Agricultural Science, 27*, 77-83.

Ullah, M.Z., Bashar, M.K., Bhuiyan, M.S.R., Khalequzzaman, M., & Hasan, M.J., (2011). Interrelationship and cause-effect analysis among morphophysiological traits in biroin rice of Bangladeshi. *International Journal of Plant Breeding and Genetics*, *5*, 246-254

Zou, G.H., Mei, H.W.H., Liu, Y., Liu, G.L., Hu, S.P., Yu, X.Q., Li, M.S., Wu, J.H., & Luo, L.J. (2005). Grain yield responses to moisture regimes in a rice population: association among traits and genetic markers. *Theoretical and Applied Genetics*, *112,* 106-113.

Received: November 17, 2023
Accepted: May 7, 2024

ANALIZA GENOTIPSKIH I FENOTIPSKIH KORELACIJA I KOEFICIJENATA PUTA KOD 40 GENOTIPOVA PLANINSKOG PIRINČA (*ORYZA SATIVA* L.) U KIŠNIM OBLASTIMA U REGIONIMA DRŽAVA OJO I OGUN U NIGERIJI

Dans K. Johnson[1], Emmanuel O. Idehen[1,2] i Isaac O. Abegunde[1*]

[1]Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[2]Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

R e z i m e

Održiva proizvodnja pirinča u planinskim staništima zavisi od postizanja većih prinosa. Ova studija koristi korelacionu analizu i analizu koeficijenata puta kako bi se identifikovali kriterijumi ključnih osobina za unapređenje prinosa pirinča kod planinskih genotipova. Studija obuhvata dva vegetaciona perioda sa 40 genotipova. Genotipska korelaciona analiza pokazuje jaku pozitivnu korelaciju efikasnog bokorenja sa brojem metlica i prinosom. Naročito pokazuje značajne negativne korelacije sa masom 1000 zrna i širinom lista na različitim lokacijama i u različitim sezonama. Pored toga, fenotipske procene ističu značajnu pozitivnu korelaciju između prinosa i broja metlica. Dalje, analiza puta pokazuje da broj metlica održava značajno pozitivnu povezanost sa prinosom na nivou značajnosti od 5%. Osim toga, analiza direktnih i indirektnih genotipskih uticaja ističe značaj broja stabala, efikasnog bokorenja i broja metlica, od kojih svi pokazuju izuzetne i pozitivne korelacije sa prinosom, postižući statističku značajnost na nivou i od 5% i 1%. Da bi se poboljšao prinos zrna pirinča, genotip mora imati povećan broj ključnih osobina po biljci, uključujući povećan broj metlica, povećanu dužinu metlice, veći broj stabala, veću dužinu stabla, povećan broj efikasnih bokora, rani početak cvetanja, ubrzano sazrevanje i povećanu dužinu lista. Ove karakteristike su ključne determinante koje značajno doprinose ukupnom prinosu zrna u gajenju pirinča i od suštinskog su značaja za održivo unapređenje pirinča u ovim agroekološkim uslovima.

Ključne reči: genetička raznovrsnost, unapređenje pirinča, prinos, karakteristike povezane sa prinosom, interakcije genotipa sa okruženjem.

Primljeno: 17. novembra 2023.
Odobreno: 7. maja 2024.

[*]Autor za kontakt: e-mail: ioabegunde@gmail.com

INSTRUCTIONS FOR AUTHORS

MANUSCRIPT SUBMISSION

By submitting a manuscript authors warrant that their contribution to the Journal is their original work, that it has not been published before, that it is not under consideration for publication elsewhere, and that its publication has been approved by all co-authors, if any, and tacitly or explicitly by the responsible authorities at the institution where the work was carried out.
Authors are exclusively responsible for the contents of their submissions, the validity of the experimental results and must make sure that they have permission from all involved parties to make the data public.
Authors wishing to include figures or text passages that have already been published elsewhere are required to obtain permission from the copyright holder(s) and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.
Authors must make sure that all only contributors who have significantly contributed to the submission are listed as authors and, conversely, that all contributors who have significantly contributed to the submission are listed as authors.
The registration of the authors and the submission of the papers should be done via the following link: http://aseestant.ceon.rs/index.php/jas/user
Manuscripts are to be pre-evaluated at the Editorial Office in order to check whether they meet the basic publishing requirements and quality standards. They are also screened for plagiarism.
Authors will be notified by email upon receiving their submission. Only those contributions which conform to the following instructions can be accepted for peer-review. Otherwise, the manuscripts shall be returned to the authors with observations, comments and annotations.

MANUSCRIPT PREPARATION

Authors must follow the instructions for authors strictly, failing which the manuscripts would be rejected without review.
The manuscript should be written in MS-Word in .doc, .docx, format. Font Times New Roman, font size 12, single spacing, margin 2.5 cm should be used when writing the paper. Page numbering should be avoided.
Original scientific paper - The paper should report the unpublished results of original research. This paper should occupy 6 to 12 pages.
Review article - The article which contains original, detailed and critical review of research problem or area where the author has made a certain contribution, noticed by auto citation (at least 10). This article should occupy 15 to 20 pages.
Preliminary communication - Original research paper of full format, small-scale or preliminary character. It should occupy 2 to 6 pages.

The obligatory parts of each Original scientific paper and Preliminary communication are the following: Title of the paper, Name(s) of author(s), Complete postal address(es) of affiliations, Abstract, Key words, Introduction, Material and Methods, Results and Discussion, Conclusion, Acknowledgements, References and Summary in Serbian (if manuscript is submitted in English and vice versa). The obligatory parts of each Review article are the following: Title of the paper, Name(s) of author(s), Complete postal address(es) of affiliations, Abstract, Key words, Introduction, Analysis-discussion of a certain topic, Conclusion, References and Summary in Serbian (if manuscript is submitted in English and vice versa). If manuscript is written in English British version is preferred.

Title of the paper

The title of the paper should describe the content of the paper as accurately and concisely as possible. Authors are recommended to use words in the title which are suitable for indexing and browsing purposes. The title should be centred and written in capital letters. If the paper has already been announced at certain meeting as an oral presentation, under the same or similar title, the datum should be stated on it at the bottom of the first page, after the data of the corresponding author.

Authors' Names

First name, middle initial(s) and last (family) name of all authors, in the original form, should be provided. The names should be written below the title, in lower-case letters, centred and bolded. If several different affiliations need to be mentioned, using the command "insert footnote", consecutive numerals should be placed as the superscript after the respective author's name. The corresponding author should be designated with an asterisk as the superscript, after the last (family) name, and his/her e-mail address should be given under the line, at the bottom of the first page of the paper.

Authors' Affiliations

The full name and address of the institution where the author is employed should be provided. It should be centred and written immediately after the author's name. If authors belong to different institutions, the numerals should be placed as the superscript before the name of institution to provide information on the institution where each of the stated authors is employed.

Abstract

The abstract is a short informative review of the content of the paper, which should enable the reader to estimate its relevance easily and accurately. It is in the interests of the author that the abstract contains terms used for indexing and browsing purposes. The references should not be given in the abstract. The abstract should include the aim of research, the methods, the results and the conclusion. It should contain between 200 and 250 words and be placed between the name of the authors' affiliations and key words. The title of the abstract should be bolded and indented pressing the tab key. The colon should be used after the title of the abstract, and then the text of the abstract should follow without any indentation.

Key words
Key words are terms or phrases which describe best the content of the article for the needs of indexing and browsing purposes. The number of key words should be 3 to 10. They should appear below the abstract. The title of key words should be bolded and indented by pressing the tab key. The colon should be used after the title, and then the list of key words in lower-case letters should be given with the full stop at the end. Key words should be provided in Serbian and English after abstract on both languages.

Introduction
The introduction should contain all the relevant information on past researches according to the stated problem and what can be achieved by further research. Reviewing the references, the author and the year should be provided, and the mentioned author should be cited in References. The title of the introduction should be centred and bolded, written in lower-case letters, below which using one line spacing, the text of the introduction should follow, justified. Each new paragraph should be indented pressing the tab key. These rules should be applied to all parts of the paper.

Material and Methods
The material and methods should be clearly outlined explaining all applied procedures in the paper. Generally known methods should be presented briefly, and a detailed explanation should be given if there is a deviation from previously published procedures. Papers, which have an experimental character, should provide the way of statistical data processing. This part, as well as the part Results and Discussion, if needed, may comprise certain subparts, too.

Results and Discussion
In the part Results and Discussion data obtained on the basis of observation and conducted experiments should be interpreted. In the comment of the results, references should be quoted at the end of the paper, providing the comparison between the obtained results and previous knowledge of the certain area.

Conclusion
All relevant items achieved in the researched area should be mentioned in the conclusion. Listing of all results with repetition of numbers previously specified in Results and Discussion should be avoided. Conclusion should not contain references.

Acknowledgements
Acknowledgements should contain the title and the number of the project that is the title of the program within which the paper was written, as well as the name of the institution which financed the project or program. It should be placed between the conclusion and references.

References

The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

The references, cited in the text should be stated in the list of references in the original form, alphabetically, without numbering. If a greater number of publications of the same author is cited, then the papers where the author is the single author should first be cited and then the publications of the same author with one and then with more co-authors. If a considerable number of publications appear in any of the above mentioned categories, they should be listed chronologically (1997, 2002, 2006, etc.), and if a great number of publications is of the same year then the letters are added (2005a, 2005b, 2005c, etc.). References entry should contain: the last (family) name of the author, the first letter of the author's name, the year of publishing in the brackets, the title of the paper, the title of the journal, the volume and the number of pages (the first-the last). When the book is cited, the publisher and place of publishing should be given. The lines of each reference entry should be indented after the first line. APA - Publication Manual of the American Psychological Association citation style is used in this journal.

The examples of listing references are the following:

Periodicals

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Book chapter

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R. M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Proceedings
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon, France.

Thesis
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Report
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Web site
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Summary
The summary in Serbian is given at the end of the paper and should comprise 200 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

Tables
Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

Illustrations
All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

diagram should be given. The photographs must be of high quality so that they can technically be well reproduced. They should be submitted in "TIF" or "JPG" format, and they will be printed in black and white. The title of the illustration should be justified, with a full stop at the end, single spaced from the illustration and given below it. Each illustration should be mentioned in the text.

Abbreviations and units
Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature
The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

Formulae
All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

The corresponding author will be sent a free copy of the journal after it has been published.
All future associates are asked to prepare the paper according to the given instructions in order to facilitate the work of the Editorial Board. Unless the paper is prepared according to the given instructions it will not be accepted for the prospective publishing.

Editorial Board of the Journal
Journal of Agricultural Sciences

UPUTSTVO AUTORIMA

SLANJE RUKOPISA

Prilikim podnošenja rukopisa autori garantuju da rukopis predstavlja njihov originalan doprinos, da nije već objavljen, da se ne razmatra za objavljivanje kod drugog izdavača ili u okviru neke druge publikacije, da je objavljivanje odobreno od strane svih koautora, ukoliko ih ima, kao i, prećutno ili eksplicitno, od strane nadležnih tela u ustanovi u kojoj je izvršeno istraživanje.
Autori snose svu odgovornost za sadržaj ponesenih rukopisa, kao i validnost eksperimentalnih rezultata, i moraju da pribave dozvolu za objavljivanje podataka od svih strana uključenih u istraživanje.
Autori koji žele da u rad uključe slike ili delove teksta koji su već negde objavljeni dužni su da za to pribave saglasnost nosilaca autorskih prava i da prilikom podnošenja rada dostave dokaze da je takva saglasnost data. Materijal za koji takvi dokazi nisu dostavljeni smatraće se originalnim delom autora.
Autori garantuju, da su kao autori navedena samo ona lica koja su značajno doprinela sadržaju rukopisa, odnosno da su sva lica koja su značajno doprinela sadržaju rukopisa navedena kao autori. Registracija autora i prijava radova se vrši preko linka: http://aseestant.ceon.rs/index.php/jas/user
Pri prijavi rada autori treba da navedu podatke za kontakt (ime i prezime, ustanovu i E-mail adresu) najmanje tri potencijalna recenzenta. Oni treba da budu eksperti iz date oblasti istraživanja koji će obezbediti objektivnu procenu rada. Predloženi recenzenti ne bi trebalo da budu iz iste institucije iz koje su i autori rada.
Nakon prijema, rukopisi prolaze kroz preliminarnu proveru u redakciji kako bi se proverilo da li ispunjavaju osnovne kriterijume i standarde. Pored toga, proverava se da li su rad ili njegovi delovi plagirani.
Autori će o prijemu rukopisa biti obavešteni elektronskom poštom. Samo oni rukopisi koji su u skladu sa datim uputstvima biće poslati na recenziju. U suprotnom, rukopis će, sa primedbama i komentarima, biti vraćen autorima.

UPUTSTVO ZA PRIPREMU RUKOPISA

Autori su dužni da se pridržavaju uputstva za pripremu radova. Rukopisi u kojima ova uputstva nisu poštovana biće odbijeni bez recenzije.

Za obradu teksta treba koristiti program MS-Word. Rukopise treba slati u jednom od sledećih formata .doc, .docx, koristiti font Times New Roman, veličina 12, jednostruki prored, margine 2,5 cm. Strane ne treba numerisati.
Originalan naučni rad – Rad koji sadrži prethodno neobjavljivane rezultate sopstvenih istraživanja. Obim ovog rada treba da iznosi od 6 do 12 strana.
Pregledni rad – Rad koji sadrži originalan, detaljan i kritički prikaz istraživačkog problema ili područja u kome je autor ostvario određeni doprinos, vidljiv na osnovu autocitata (najmanje 10). Obim ovog rada treba da iznosi od 15 do 20 strana.

Prethodno saopštenje – Originalan naučni rad punog formata, ali manjeg obima ili preliminarnog karaktera (od 2 do 6 strana).
Obavezna poglavlja svakog originalnog naučnog rada i prethodnog saopštenja su sledeća: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, materijal i metode, rezultati i diskusija, zaključak, zahvalnica, literatura i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Pregledni rad mora da sadrži: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, analizu-diskusiju određene teme, zaključak, literaturu i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Ako su radovi na engleskom jeziku, prednost se daje britanskoj varijanti ovog jezika.

Naslov rada
Naslov rada treba što vernije da opiše sadržaj rada i da ima što manje reči. U interesu je autora da se u naslovu koriste reči prikladne za indeksiranje i pretraživanje. Naslov se piše velikim slovima i centrirano. Ako je rad prethodno bio izložen na nekom skupu u vidu usmenog saopštenja, pod istim ili sličnim naslovom, podatak o tome treba navesti pri dnu prve stranice, posle podataka autora za kontakt.

Imena autora
Navodi se puno ime, srednje slovo i prezime svih autora, u originalnom obliku. Imena se pišu ispod naslova, malim slovima, centrirano i boldovano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu, iza prezimena, označiti ustanovu u kojoj radi svaki autor. Autor za kontakt označava se zvezdicom u superskriptu, iza prezimena, komandom „insert footnote“, a njegova e-mail adresa navodi se ispod crte pri dnu prve stranice članka.

Naziv ustanove autora
Navodi se pun naziv i adresa ustanove u kojoj je autor zaposlen. Ispisuje se neposredno nakon imena autora, centrirano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu ispred institucije označava se ustanova u kojoj je zaposlen svaki od navedenih autora.

Sažetak
Sažetak je kratak informativni prikaz sadržaja članka koji čitaocu omogućava da brzo i tačno odredi njegovu relevantnost. U interesu je autora da sažetak sadrži termine koji se koriste za indeksiranje i pretraživanje. Sažetak ne sme da sadrži reference. Sastavni delovi sažetka su cilj istraživanja, metode, rezultati i zaključak. Sažetak treba da ima od 200 do 250 reči. Reč „Sažetak“ piše se boldovano i uvlači jednim tabulatorom, nakon čega slede dve tačke, a zatim tekst sažetka.

Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim

tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod

Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode

Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

Rezultati i diskusija

U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

Zaključak

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

Zahvalnica

Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

Literatura

Poglavlje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van

zagrade vrši se zarezom (,) a u zagradi tačkom i zarezom (;). Ako se citiraju dva ili više rada istog autora oni moraju biti poređani prema hronološkom redu (1997, 2002, 2006, itd.). Ukoliko se određeni autor pojavljuje nekoliko puta u istoj godini, dodaju se slova (2005a, b, c, itd.). Citate ličnih komunikacija i neobjavljenih podataka treba izbegavati, osim ako je to apsolutno neophodno. Takvi citati bi trebali da se pojave samo u tekstu (npr. Brown, lična komunikacija), ali ne i u spisku referenci.
Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Rezime
Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 200 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmaknuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod

uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation". Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

Nakon objavljivanja rada, autoru za kontakt će biti poslat jedan primerak časopisa.
Mole se svi budući saradnici da rad pripreme prema datom uputstvu, kako bi olakšali rad redakcije časopisa. Ukoliko se rad ne pripremi po navedenom uputstvu neće biti prihvaćen za objavljivanje.

Redakcioni odbor časopisa
Journal of Agricultural Sciences

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

63

JOURNAL of Agricultural Sciences / editor-in-chief Snežana Oljača. - [Štampano izd.]. - Vol. 44, no. 1 (1999)- . - Belgrade : University of Belgrade, Faculty of Agriculture, 1999- (Belgrade-Zemun : Faculty of Agriculture). - 24 cm

Tromesečno. - Je nastavak: Review of Research work at the Faculty of Agriculture = ISSN 0354-3498. - Drugo izdanje na drugom medijumu: Journal of Agricultural Sciences (Belgrade. Online) = ISSN 2406-0968
ISSN 1450-8109 = Journal of Agricultural Sciences (Belgrade)
COBISS.SR-ID 169380871