Original Research Article

Upshot of biopolymer synthesized from Tamarind Seed Polysaccharide (TSP) on seed physiological and biochemical parameters of maize var. COH(M)8

ABSTRACT

The experiment was conducted in Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2021. Biopolymer was synthesized from Tamarind Seed Polysaccharide (TSP) and added with different additives and coated the maize seeds to know its effect on physiological and biochemical parameters. The seeds were given with four treatments *viz.,* T_0- control (untreated seed), T_1- biopolymer (B.P) @ 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) and T_3- T_2+ Ascorbic acid (0.2 g) and evaluated for seed quality parameters. The results of the present investigation revealed that T_3 was found to be significantly superior *viz.,* higher germination percentage (95%), speed of germination (31.64), seed metabolic efficiency (2.78), seedling root length (25.92 cm), seedling shoot length (15.57 cm), total drymatter production (9.41 g), vigour index I (3942), vigour Index-II (94.15). It also recorded highest value of biochemical parameters such as α-amylase (2.23), dehydrogenase (1.87 OD value), catalase (29.94) and peroxidase (7.00) activities. It reduced the abnormal seedlings (2%), dead seeds (3%), days 50% germination (2.54), mean emergence time (3.02) and pathogen infection (0.25%). The results concluded that maize seeds coated with T_3 performed better seedling establishment and could be recommended as pre sowing seed treatment under organic agriculture.

Key words: Tamarind Seed Polysaccharide, Biopolymer, Humic acid, Zimmu leaf extract, Seed germination and Vigour

1. INTRODUCTION

One of the most significant cereals in the world is maize (*Zea mays* L.). It is used as a human food, animal feed and a raw ingredient in a variety of industrial products all over the world. Maize is a high-yielding, easily digestible crop that is widely utilized in confectioneries. It's a key ingredient in the manufacture of starch, oil, protein, alcoholic drinks, food sweeteners and more recently in biofuel. It is known as the "Queen of Cereals" because of its versatility and magical properties.

Maize is a species that can adapt to a wide range of environmental circumstances, but pest and disease outbreaks can lead to lower yields and quality. Quality seed is essential for successful agriculture, as each seed must germinate quickly and create a healthy seedling, resulting in higher yields. [1]. Seed coating technology has advanced quickly over the last three decades, and it now offers a cost-effective method of seed improvement. Seed coating is the act of applying a beneficial material directly on a seed to generate a thin, homogeneous coating without changing the seed's shape or size. Seed coating has presented promising results in many crops including cereals. Seed coating with synthetic polymers has gained rapid acceptance by the seed industry. It makes room for including all the required ingredients like inoculants, protectants, nutrients, plant growth promoters, hydrophobic / hydrophilic substances, herbicides, oxygen suppliers etc.

Seed coating with polymer enhances chemical adhesion to the seed and allows for dust-free handling of treated seed [2]. The polymer coating is easy to apply, quickly diffuses, and is non-toxic to the seed during germination. The polymer coating may operate as a physical barrier, preventing inhibitors leaching from seed coverings and restricting oxygen transport to the embryo [3]. Thereby, it provides protection from the stress imposed by ageing, improves plant stand and emergence of seedlings. Polymer acts as a temperature switch and protective coating by regulating the water uptake and subsequent germination of seed [4]. Coating results in more uniform and accurate seed rate due to the smooth flow of the seed during mechanical sowing. Increase in germination can also be observed in polymer coated seed. Addition of colourant helps in visual monitoring of placement accuracy, enhance the appearance, marketability and consumer preference.

Though the polymer coating has number of benefits on seed quality parameters and agriculture, continuous use of synthetic polymer and synthetic colourant may degrade the soil quality and ultimately reduces the crop yield. Because most of the seed coating polymers are synthetic and very slow degradable in nature. Continuous using of polymer may leads to accumulation of polymer in soil profile and may causes negative effect on soil microorganisms and soil health. Eventually it may impacts on crop growth and yield, and may pollute the water body and environment. Considering this issue, we synthesized the biopolymer from tamarind seed polysaccharide and studied its effect on seed physiological and biochemical characteristics in maize.

2. MATERIALS AND METHODS

The Lab experiment was conducted to study “The Effect of biopolymer coating on seed quality parameters Hybrid Maize (*Zea mays* L.) (COH(M)8), at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2021. The treatments used were T_0- Control (untreated seed), T_1- biopolymer (B.P) @ 10g kg^{-1}, T_2- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) and T_3- B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g).

Observations

2.1. Seed physical characters

2.1.1. 100 seed weight

Seed weight was estimated by weighing 100 seeds of eight replication and the mean values were expressed in gram.

2.1.2. Seed moisture content

Moisture estimation was carried out by high constant temperature method as per ISTA protocol [5].

2.2. Seed physiological characters

2.2.1. Seed germination (%)

The germination test was carried out as per the procedure prescribed by ISTA [5] in roll towel method. The test conditions were 25$\pm$2ºC and 95$\pm$5% RH maintained in a germination room illuminated with fluorescent light. After seven days, the number of normal seedlings produced was counted and germination percentage (GP) was calculated, according to the formula:

$$\text{Germination percentage (GP)} = (Ng / Nt) \times 100$$

Where Ng is a total number of normal seedlings germinated, Nt is a total number of seeds evaluated.

2.2.2. Days for 50 per cent germination and maximum germination

In the sand media, the number seeds germinated was recorded daily up to final count and number of days required for 50 per cent germination and number of days required for maximum germination was computed according to Heydecker & Coolbear [6] and Mauromicale & Cavallaro [7] respectively.

2.2.3. Speed of germination

Numbers of seeds germinated were counted daily up to seven days at the same time of day. From the number of seeds germinated on each counting day, the speed of germination was computed adopting the formula given by Maguire [8].

2.2.4. Mean germination time

Mean germination time (MGT) was calculated according to Bailly *et al.* [9] using the formula:

$$MGT = \Sigma (Dn)/\Sigma n$$

Where, n is the number of seeds germinated on each day and D is the day of counting

2.2.5. Endosperm and embryo degradation (Seed Metabolic Efficiency)

Amount of seed respired (SMR) was calculated as

$$SMR = SDW-(SHW+RTW+RSW)$$

Where,

SDW - Seed dry weight before germination

SHW - Shoot dry weight

RTW - Root dry weight

RSW - Remaining seed dry weight

Seed Metabolic Efficiency (SME) was calculated using the formula [10]

$$SME = (SHW + RTW) / SMR$$

2.2.6. Root length and shoot length (cm)

Ten normal seedlings from the standard germination test were randomly selected and the root and shoot length was measured from the collar region to the tip of the primary root and tip of the shoot respectively. The average value was expressed in centimeter.

2.2.7. Drymatter production (g seedlings^{-10}) and total drymatter production (g) [5]

The seedlings used for growth measurement and remaining normal seedlings were placed in a butter paper cover separately and dried in shade for 24 h and then kept in an oven maintained at 85 ± 2^{o}C for 24 h. Dry weight was recorded and the mean values were expressed in g. The total drymatter production was calculated by adding dry weight of ten seedlings and remaining normal seedlings.

2.2.8. Vigour index [11]

Vigour index values were computed using the following formula and the mean values were expressed in whole number.

Vigour index I = Germination (%) x Total seedling length (cm)

Vigour index II = Germination (%) x Drymatter production (g seedling^{-10})

2.3. Biochemical parameters

2.3.1. Dehydrogenase activity (OD value)

Dehydrogenase activity was estimated as per the procedure described by Kittock and Law [12].

2.3.2. α-amylase activity (mg maltose liberated / minute)

α- amylase activity in pre germinated seeds were carried out according to the method suggested by Simpson and Naylor [13].

2.3.3. Peroxidase activity (∆ A436 / min/ g of seed)

Peroxidase activity was estimated as per the procedure described by Singh *et al.* [14].

2.3.4. Catalase activity (mMol H_2O_2 degraded / minute)

Catalase activity was measured by an assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdate [15].

2.4. Seed health

2.4.1. Pathogen infection (%)

Pathogen infection was assessed as per the protocol given by ISTA [5].

3. RESULTS AND DISCUSSION

The result revealed that, seeds coated with bio polymer (B.P) and B.P with additives shows non-significant difference for 100 seed weight and moisture content (Table 1). Polymer coating forms a very thin layer around the seeds without obscuring size and shape, hence it did not altered the seed weight. After seed coating the seeds were dried under shade for one hour, hence it did not change the moisture content significantly in coated seeds.

According to the results, all studied traits were affected by the treatments and there was completely significant difference observed among treatments. Seed physiological characters *viz.,* germination percent (95%), speed of germination (31.64), seed metabolic efficiency (2.78), seedling root length (25.92 cm), seedling shoot length (15.57 cm), drymatter production/10 seedlings (0.991 g), total drymatter production (9.41 g), vigour index-I (3942), and vigor index-II (94.15) were significantly recorded maximum in T_3 whereas found lowest in control (Table 1,2 and Fig. 1,2). It significantly reduced days for 50% germination (2.54), mean emergence time (3.02), abnormal seedling (2%) and dead seeds (3%) compare to other treatments (Table 1).

The improvement in seed physiological parameters in T_3 may be due to additives such as humic acid and ascorbic acid present in the polymer formulation. Humic acid is one of bio-stimulants that are known as the organic substances which promote plant growth [16]. Humic acid improves the nutrient availability especially microelements in soils because it promotes nutrient uptake in the form of chelating agent. Moreover, humic substances may increase root growth in a similar manner to auxins [17, 18]. The present results similar to the findings of Asgharipour and Rafiei [19] and Basalma [20] who reported that seed treated with HA recorded maximum germination, seedling length, seedling fresh weight, seedling dry weight and vigour index in barley and safflower respectively. Likewise, HA seed treatment increased the shoot fresh and dry weight of seedlings in tomato [21], maize [22], pea [23], wheat [24], cucumber, squash and marigold [25].

The activity mechanism of humic acid in promoting plant growth is not fully understood, and the beneficial effects to plants are difficult to comprehend due to its chemical heterogeneity [26,27]. The most established explanations for the beneficial effects of HA are related to their positive influence on ion transport, which improves cell permeability, thereby affects absorption. They also promote increased respiration and speed of enzymatic reactions of the Krebs cycle, resulting in increased ATP production, altering directly plant metabolism and consequently may influence growth and development [28,29]. The increase in absorption rates can be explained by the activation of ATPase present in the plasma membrane [30], acting on two mechanisms essential to plant growth, through the energy supply to the secondary systems in the translocation of ions and by increasing plasticity of the cell wall, thus allowing cell growth and division [31].

The reason for improved seed physiological parameters in T_3 may also be due to ascorbic acid present in the polymer formulation. Ascorbic acid (AsA), also known as ascorbate or vitamin C, is a low molecular weight water-soluble antioxidant both in plants and animals. And AsA is a universal non-enzymatic antioxidant having a substantial potential of not only scavenging reactive oxygen species (ROS), but also modulating many fundamental functions in plants both under stress and nonstress conditions [32,33,34]. Burguieres *et al.* [35] and Chen *et al.* [36] reported that seeds treated with AsA increased the germination, seedling length, fresh weight and dry weight in pea and alfalfa respectively.

Table 1. Effect of biopolymer and additives on stand establishment traits of maize

Treatments	100 seed weight (g)	Seed moisture content (%)	Abnormal seedling (%)	Dead seeds (%)	Days for 50 per cent germination	Speed of germination	Mean emergence time
T_0	31.27	10.22	5	5	3.16	27.31	3.37
T_1	31.44	10.24	5	5	3.14	27.66	3.34
T_2	31.43	10.23	2	5	2.62	28.42	3.08
T_3	31.44	10.22	2	3	2.54	31.64	3.02
Mean	**31.40**	**10.23**	**4**	**5**	**2.87**	**28.76**	**3.20**
SEd	**0.26**	**0.06**	**0.03**	**0.06**	**0.04**	**0.40**	**0.03**
CD (P=0.05)	**0.55**	**0.13**	**0.06**	**0.12**	**0.08**	**0.85**	**0.06**

Table 2. Effect of biopolymer and additives on seedling vigour parameters of maize

Treatments	Seed metabolic efficiency	Shoot length (cm)	Root length (cm)	Dry matter production (g/10 seedlings)	Total dry matter production (g)	Vigour index II
T_0	2.24	12.85	23.48	0.908	8.17	81.72

T_1	2.26	13.14	23.85	0.912	8.21	82.08
T_2	2.65	15.14	25.65	0.956	8.89	88.91
T_3	2.78	15.57	25.92	0.991	9.41	94.15
Mean	**2.48**	**14.18**	**24.73**	**0.942**	**8.67**	**86.72**
SEd	**0.02**	**0.13**	**0.39**	**0.01**	**0.06**	**1.03**
CD (P=0.05)	**0.04**	**0.28**	**0.83**	**0.02**	**0.13**	**2.18**

T_0 T_3

Fig. 1. Effect of biopolymer with additives on seed germination and seedling growth of maize

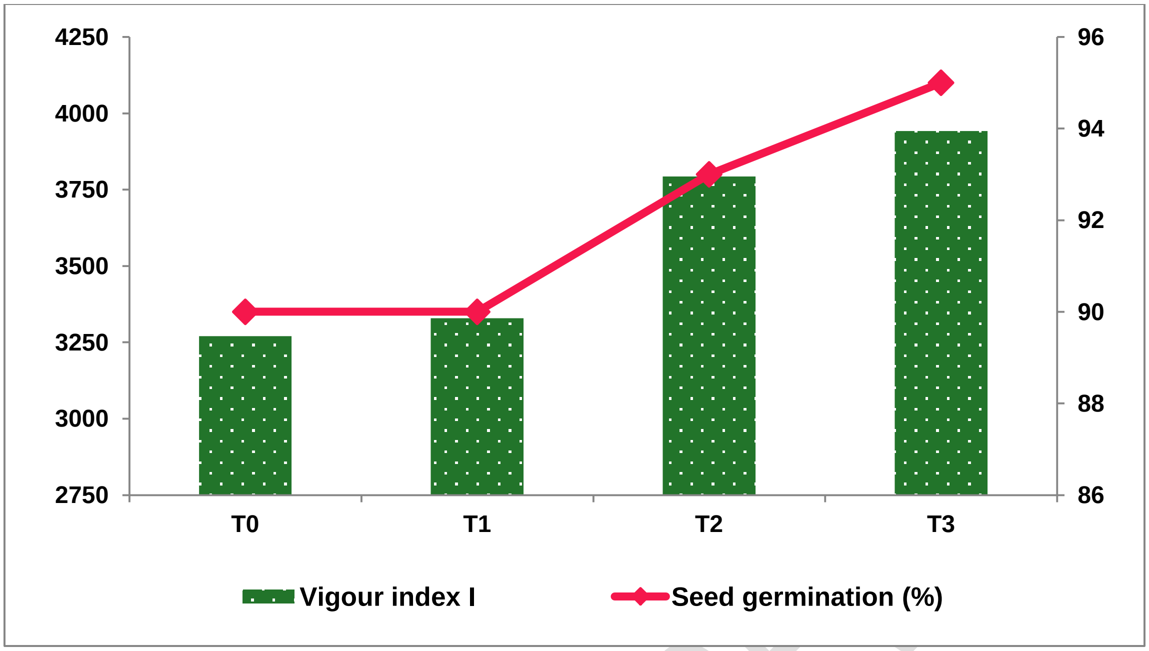

Fig. 2. Effect of biopolymer with additives on seed germination and seedling vigour index I of maize

Among the treatments T_3 recorded significantly higher levels of enzymatic activities *viz.,* α-amylase (2.23 mg maltose/min), dehydrogenase (1.87 OD value), catalase (29.94 mMol H_2O_2 / min) and peroxidase (7.00 ∆ A436 /min/g) compared to other treatments (Table 3). The result similar to the findings of Burguieres *et al.* [35] who stated that seeds treated with AsA increased the guaiacol peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) activities in pea. Similarly, Chen *et al.* [36] reported that seeds treated with AsA increased the α-amylase and protease activities in alfalfa seeds.

The results shows that the seeds treated with T_2 and T_3 significantly reduced the pathogen infection (0.25%) compared to T_0 and T_1 (1.25 and 1.50% respectively) (Table 3). This may be the reason for reduced dead seed percentage in T_2 and T_3. Reduced pathogen infection is due to added antimicrobial agent (zimmu leaf extract) in the polymer. Satya *et al.* [37] found that the leaf extract of zimmu showed the maximum antifungal activity against *Rhizoctonia solani* and it also effective in inhibiting the growth of other fungal and bacterial pathogens *viz., Aspergillus flavus, Alternaria solani, Curvularia lunata, Xanthomonas campestris* pv. *Malvacearum, X. oryzae* pv. *oryzae,* and *X. oxonopodis* pv. *citri*. Thus the study indicates that the physiological and biochemical improvement in T_3 is due to synergetic effect of humic acid and ascorbic acid present in the polymer.

Table 3. Effect of biopolymer and additives on biochemical parameters and pathogen infection of maize

Treatments	α-amylase activity (mg maltose min^{-1})	Dehydrogenase activity (OD value)	Catalase activity (µg H_2O_2/min/mg protein)	Peroxidase activity (ΔOD 430 mg^{-1} min^{-1})	Pathogen infection (%)
T_0	2.17	1.76	28.16	6.58	1.25
T_1	2.17	1.78	28.18	6.59	1.50
T_2	2.18	1.80	28.53	6.59	0.25
T_3	2.23	1.87	29.94	7.00	0.25
Mean	**2.19**	**1.80**	**28.70**	**6.69**	**0.81**
SEd	**0.03**	**0.02**	**0.17**	**0.05**	**0.009**
CD (P=0.05)	**0.07**	**0.03**	**0.37**	**0.12**	**0.020**

4. CONCLUSION

Biopolymer and additives had a significant effect on physiological and biochemical seed quality traits in maize. T_3 improved the stand establishment such as seed germination, speed of germination, seed metabolic efficiency, shoot length, root length, drymatter production, vigour index I and vigour index II compared to other treatments. It also increased the dehydrogenase, α- amylase, catalase and peroxidase activities. It reduced the days for 50 % germination, mean germination time, abnormal seedlings, dead seeds and pathogen infection compared to control. Thus the study highlighted that seeds coated with B.P (10g) + Humic acid (0.3g) + Zimmu leaf extract (0.5 ml) + ascorbic acid (0.2 g) improved the seed quality characteristics in maize.

REFERENCES

1. Ananthi M, Selvaraju P, Srimathi P. Effect of seed treatment on seed and seedling quality characters in redgram cv. CO (Rg) 7. International Journal of Science and Nature. 2015;6(2): 205-208.
2. Ekebafe LO, Ogbeifun DE, Okieimen FE. Polymer applications in agriculture. Biokemistri. 2011;23(2):81-89. French.
3. Kumar M, Kumar A, Kumar R, Yadav SK, Yadav R, Kumar J. Effect of seed enhancement treatment on field performance of chickpea (*Cicer arietinum* L.). Journal of Applied and Natural Science. 2014;7(2):557–561.
4. John SS, Bharathi A, Natesan P Raja K. Effect of polymer coating on germination and seedling vigour in maize cv. Co 1. Karnataka Journal of Agricultural Sciences. 2005;18(2):343-348.

5. ISTA. International Rules for Seed Testing. International Seed Testing Association, Switzerland. 2013.
6. Heydecker W, Coolbear P. Seed treatments for improved performance survey and attempted prognosis. Seed Science and Technology. 1977;5:353–425.
7. Mauromicale G, Cavallaro V.. Effects of seed osmopriming on germination of tomato at different water potential. Seed Science and Technology. 1995;23:393-403.
8. Maguire JD. Speed of Germination - Aid In Selection And Evaluation for Seedling Emergence And Vigor1. Crop science. 1962;2(2):176-177.
9. Bailly C, Benamar A, Corbineau F, Come D. Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. Seed Science Research. 2000;10:35–42.
10. Rao DG, Sinha SK. Efficiency of Mobilization of Seed Reserves in Sorghum Hybrids and Their Parents as Influenced by Temperature. Seed Research. 1993;21:97-100.
11. Abdul-Baki AA, Anderson JD. Vigour deterioration of soybean seeds by multiple criteria. Crop Science. 1973;13:630-633.
12. Kittock DL, Law AG. Relationship of seedling vigour to respiration and tetrazolium chloride reduction by germinating wheat seeds. Agronomy Journal. 1968;60:286-288.
13. Simpson G, Naylor J. Dormancy studies in seed of *Avena fatua*: 3. a relationship between maltase, amylases, and gibberellin. Canadian Journal of Botany. 1962.40(12): 1659-1673.
14. Singh M, Bhalla PL, Malik C. Activity of Some Hydrolytic Enzymes in Autolysis of the Embryo Suspensor in *Tropaeolum majus* L. Annals of Botany. 1980;45(5):523-527.
15. Goth L. A simple method for determination of serum catalase and revision of reference range. Acta. 1991;196:143-152.
16. Chen Y, Magen H, Riov J. Humic substances originating from rapidly decomposing organic matter. Proc Int Meet. 1994;427- 443 (Chem Abst 121: 229).
17. Donnell RW. The auxin-like effects of humic preparations from leonardite. Soil Science. 1973;11(6):106–112
18. Khattab MM, Shaban AE, El-Shrief AH, Mohamed AS. Effect of humic acid and amino acids on pomegranate trees under deficit irrigation. I: Growth, flowering and fruiting. Journal of Horticultural Science and Ornamental Plants. 2012;4:253–259
19. Asgharipour MR, Rafiei M. The Effect Of different Concentrations Of humic Acid on seed Germination Behavior and Vigor of barley. Australian Journal of Basic and Applied Sciences. 2011;5(12):610-613.
20. Basalma D. Effects of Humic Acid on The Emergence and Seedling Growth of Safflower Varieties (*Carthamus tinctorius* L.). Turkish Journal Of Agricultural And Natural Sciences. 2015;2(2):152–156.

21. David PP, Nelson PV, Sanders DC. A humic acid improves growth of tomato seedlings in solution culture. Journal of Plant Nutrition. 1994;17:173–184.
22. Tan KH, Nopamornbodi V. Effect of different humic acids on nutrient content and growth of corn. Plant and Soil. 1979;51:283–287.
23. Vaughan D. A possible mechanism for humic acid action on cell elongation in root segments of *Pisum sativum* under aseptic conditions. Soil Biology and Biochemistry. 1974;6:241–247.
24. Malik KA, Azam F. Effect of humic acid on wheat (*Triticum aestivum* L.) seedling growth. Environment and Experimental Botany. 1985;25:245–252.
25. Hartwigsen JA, Evans MR. Humic Acid Seed and Substrate Treatments Promote Seedling Root Development. Hortscience. 2000;35(7):1231–1233.
26. Vesela L*, et al*. Structure and properties of natural humic substances of the oxihumolite type. Chemické Listy. 2005;99(1)711-717. French.
27. Muscolo A. *et al.* Biological activity of humic substances is related to their chemical structure. Soil Science Society of America Journal. 2007;71(1):75-85.
28. Zandonadi DB, Canellas LP, Facanha AR. Indolacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H+ pumps activation. Planta Berlin. 2007;225(6):1583-1595.
29. Chen Y, Clapp CE, Magen H. Mechanisms of plant growth stimulation by humic substances: the role of organoiron complexes. Soil Science and Plant Nutrition. 2004;50(2):1089-1095.
30. Canellas LP, Facanha AR. Chemical nature of soil humified fractions and their bioactivity. Pesquisa Agropecuária Brasileira. 2004;39(3):233-240. French.
31. Rodda MRC, *et al.* Estímulo no crescimento e na hidrolise de ATP em raizes de alface tratadas com humatos de vermicomposto. Revista Brasileira de Ciencia Solo Vicosa. 2006;30(4):649-656. French.
32. Kumar S, Kaur R, Kaur N, Bhandhari K, Kaushal N, Gupta K, *et al*. Heat-stress induced inhibition in growth and chlorosis in mungbean (*Phaseolus aureus* Roxb.) is partly mitigated by ascorbic acid application and is related to reduction in oxidative stress. Acta Physiol Plant. 2011; 33:2091–2101. https://doi. org/10.1007/s11738-011-0748-2.
33. Farooq M, Irfan M, Aziz T, Ahmad I, Cheema S. Seed priming with ascorbic acid improves drought resistance of wheat. Journal of Agronomy and Crop Science. 2013;199:12–22.
34. Billah M, Rohman M, Hossain N, Uddin MS. Exogenous ascorbic acid improved tolerance in maize (*Zea mays* L.) by increasing antioxidant activity under salinity stress. African Journal of Agricultural Research. 2017;12:1437–1446.
35. Burguieres E, McCue P, Kwon Y, Shetty K. Effect of vitamin C and folic acid on seed vigour response and phenolic-linked antioxidant activity. Bioresource Technology. 2007;98:1393–1404

36. Chen Z, Xin-long Cao, Jun-peng Niu D. Effects of exogenous ascorbic acid on seed germination and seedling salt-tolerance of alfalfa. PLOS ONE. 2021;16(4):1-16.
37. Satya VK, Radhajeyalakshmi R, Kavitha K, Paranidharan V, Bhaskaran R, Velazhahan R. In vitro antimicrobial activity of zimmu (*Allium sativum* L. x *Allium cepa* L.) leaf extract. Archives of Phytopathology and Plant Protection. 2005;38(3):185–192.