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Standardisation of seed priming treatments with Pyroligneous acid (PA) to enhance seed germination and seedling growth under saline stress in Greengram (*Vigna radiata* L.)

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Abstract
Seed germination and seedling growth are adversely affected by salinity. Seed priming is a low-cost and feasible technology to enhance seed germination and seedling vigour under stress conditions. Pyroligneous acid (PA) is a bio-stimulant, rich in bioactive components which improves crop growth and tolerance under salinity condition. The effect of seed priming treatments at various concentrations of PA (1:200, 1:300, 1:400, 1:500, and 1:600) on seed germination and seedling growth parameters of greengram under saline (100mM NaCl) stressed growing media were investigated. Severe reduction in germination percentage of greengram seeds was observed in non-primed seeds grown under saline stress condition (T_2 - 41%) when compared to control (T_1 - 89%). Among these treatments, seeds primed with PA at 1:500 dilution recorded higher germination percentage (64%), root length (17 cm), shoot length (13.92 cm), seedling dry weight (0.179 mg 10 seedling^{-1}) and Vigour Index (VI-I 2133 and VI-II 12.32) when compared to control under saline stress conditions. The results clearly indicated the effect of seed priming treatments in alleviating the negative effect of saline stress in germinating seedlings.

Keywords: Pyroligneous acid, greengram, seed priming, saline stress

Introduction
Salt stress is one of the major abiotic threats of agriculture worldwide and significantly reduces crop yield in affected areas. Around 6.74 million ha area in India is salt-affected. Estimates suggest that every year nearly 10% additional area is getting salinized, and by 2050, around 50% of the arable land would be salt-affected. Saline soils occupy 44% area covering 12 states and one Union Territory, while sodic soils occupy 47% area in 11 states of the country (Kumar and Sharma, 2020) [1]. Excess salts in the root zone hinder plant roots from withdrawing water from surrounding soil. This lowers the amount of water available to the plant, regardless of the amount of water actually present in the root zone. Seed germination is a complex cellular and physiological processes, begins with water imbibition, which strongly depends on growing media water potential (Bewley *et al.*, 2012) [2]. Soil salinity influences seed germination by establishing an osmotic potential outside the seed, which prevents water intake, or by toxic effects of Na^+ and Cl^- ions on sprouting seed (Khajeh *et al.*, 2003) [3]. The major cause of salinity damage is a change in water relationship produced by high salt deposition in intercellular gaps (Zhang *et al.*, 2006) [4]. Thus, plants tolerance to salt during germination and early seedling stages is critical for species establishment in a saline environment. Seedlings are the most vulnerable stage of a plant's life cycle, and when and where seedling development begins is determined by germination (Kaymakanova, 2009) [5].
Seed priming is a seed enhancement technique in which seeds are partially hydrated with natural and synthetic compounds before germination, which allows sufficient hydration and advancement of metabolic processes but prevents germination. Seed priming ensures increased and uniform germination by reducing the imbibition time (Brocklehurst *et al.*, 2008) [6], increasing the pre-germinative enzyme activation, increasing metabolite production (Hussain *et al.*, 2016) [7], repairing the damaged DNA (Farooq *et al.*, 2009) [8], and regulating osmosis. It also repairs the damage of aged seeds (Butler *et al.* 2009) [9] or seeds exposed to abiotic stresses such as salinity. In plant defence, priming is defined as a physiological process by which a plant prepares to respond to imminent abiotic stress more quickly or aggressively.

Priming involves prior exposure to elicitors which brings a cellular state that arrest the harmful effects of abiotic stress, and plants raised after priming are more tolerant to abiotic stress (Arun *et al,* 2022) [10].

Pyroligneous acid (PA) or wood vinegar is a dark brown solution obtained as a by-product of wood carbonization. It contains over 200 components, such as acids, alcohols, phenols and neutrals (Yatagai *et al.,* 1998) [11]. The chemical composition of PA is complex, which makes it more challenging to fully understand its biostimulatory mechanisms in plants. The presence of Karrinkins in PA plays a significant role in seed germination and seedling growth (Dixon *et al.,* 2009 & Umehara *et al.,* 2008) [12, 13]. Apart from karrinkins, PA is rich in other organic compounds including organic acids and several derivates of alcohol that could influence seed germination and seedling growth (Grewal *et al.,* 2018) [14]. Alcohol has been reported to induce seed germination in several crop species (Salehi *et al.,* 2008) [15]. One postulated mechanism of alcohol stimulation of seed germination is its involvement in the activation of the Kreb's cycle and glycolysis for energy generation during seed germination and emergence (Miyoshi and Sato, 1997) [16].

Recent studies showed that PA enhances seed germination rate, vegetative and reproductive growth of several plants species (Shan *et al.,* 2018; Mu *et al.,* 2003) [17, 18]. But, the concentration of PA varied with crop species. In wheat, a significant increase in germination and growth was observed at 1:900 (v/v) PA (Wang *et al.,* 2019) [19]; whereas in rice seedling vigor, shoot and root length and fresh weight were improved by 1:500 (v/v) PA (Kulkarni *et al.,* 2006) [20]. Several studies proved that PA has high ROS-scavenging activities, reducing power, and anti-lipid peroxidation capacity (Loo *et al.,* 2007; Wei *et al.,* 2010) [21, 22]. These suggest that PA has the potential to enhance plant growth and productivity, and can contribute significantly to plant tolerance to abiotic stress. Among the various pre-sowing treatments, seed priming is an easy, low-cost, and effective method to overcome the environmental stress problems (Ashraf and Foolad, 2005) [23]. Hence, the following study was conducted to assess the effect of PA seed priming on seed germination and seedling growth of greengram under saline stress conditions.

Material and Methods

The experiment was conducted in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2021-22. Seeds of Greengram cv. VBN 4 were obtained from the National Pulses Research Centre (NPRC), Pudukkottai, Tamil Nadu. Seeds were surface sterilised with 0.1% sodium hypochloride (NaHCl) for 2-3 minutes and washed with distilled water thoroughly. The tolerance level of greengram seeds for salinity were screened by subjecting seeds to germination test in varying concentrations of NaCl solution *viz.,* 60mM, 80mM, 100mM, 120mM, and 150mM of NaCl with control. The seeds were evaluated for germination percentage, root length and shoot length based on 50% mortality of the seedlings and the concentration for NaCl was fixed to induce saline stress.

The seeds were primed with sterilized water (T_3) and PA at 1:200 (T_4), 1:300 (T_5), 1:400 (T_6), 1:500 (T_7), and 1:600 (T_8) PA/ddH_2O (v/v). The primed seeds along with non-primed seeds (control) were subjected to germination test under saline stressed media. Then the primed seeds were evaluated for germination percentage (ISTA, 2015), root length (cm), shoot length (cm), seedling dry weight (mg 10 seedling^{-1}) and vigour index (Abdul-Baki and Anderson, 1973) [24].

The experimental treatments were arranged in a Completely Randomized Design (CRD) with four replications. Data was subjected to statistical analysis using analysis of variance (ANOVA) after transforming the percentage data to arcsine value to homogenize the variance (Panse and Sukhatme, 1985) [25]. The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance (*). If F test is non-significant, it was indicated as NS. Data were subjected to analysis of variance (ANOVA) using SPSS for Windows (SPSS®, Version 22.0. Armonk, New York, USA).

Result and Discussion

Screening of saline tolerance level using sodium chloride (NaCl) in greengram

The salinity tolerance level of greengram was screened using different concentrations of NaCl. The seedlings survival percentage decreased in all NaCl concentrations when, compared to control. The control (T_1) seeds recorded 88% germination, whereas the seeds sown in 150mM NaCl (T_6) concentration recorded only 11% germination. Similarly, Babbar and Dhingra (2007) [26] found a decline in seed germination and seedling growth rate with increase in salt concentration in mung bean cultivars. However, 50% seedling survival percentage was observed at 100mM (T_4) of saline stress (Fig. 1). The difference in the germination percentage of green gram at saline stress condition would helpful to identify the tolerance level to saline stress condition. Seedling root and shoot length were significantly affected at higher NaCl concentrations. Control (T_1) seeds recorded maximum root length (18.15 cm) and shoot length (17.12 cm) and the minimum root length (7.28 cm) and shoot length (5.70 cm) was recorded in seeds grown in 150mM NaCl (T_6) concentration. The seeds grown in 100mM NaCl (T_4) concentration recorded root length and shoot length of 9.62 cm and 7.92 cm respectively.

Root growth is more vulnerable to salt stress than shoot elongation, and the roots are also more seriously injured since they are the first organs to be stressed (Berhanu and Berhane, 2014) [27]. Furthermore, the seedling dry weight and vigour index (I & II) were higher in control (0.166 & 3112) and lower in T_6 (0.04 & 132) treatment. Increasing concentration of salt gradually decreased the seedling dry weight and vigour index in maize (Ahmed *et al.,* 2017) [28]. Based on the screening experiment the concentration of NaCl was fixed at 100mM.

Standardization of concentration of PA under saline stress

The results clearly showed a significant difference in germination, root length, shoot length, seedling dry weight and vigour index of both PA primed and non- primed seeds under saline stress conditions (Table. 1). Lei *et al.* (2018) [29] reported that PA at the correct dilution might be employed as a potential priming agent for seed germination, hence boosting crop yields. Seeds primed with PA showed higher germination percentage under saline stress conditions. Under normal condition, the seeds recorded 89% germination. Among the priming treatments, seeds primed with 1:500 PA (T_7) recorded maximum germination (64%) in saline stress condition compared to control (43%) and hydro primed (51%) seeds, followed by T_8 (1:600 PA) with 57% germination which is on par with T_6 (1:400 PA). These findings are similar to previous studies by Sparg *et al.* (2006) [30], who reported that a concentration of 1:500 PA solution enhanced seed germination percentage in maize. Aqueous PA solutions exhibit hormone-like responses in various species and interact with auxins, cytokinins, ethylene, gibberellins, and abscisic acid in different types of seeds (Daws *et al.*, 2007) [31].

The reduction in germination percentage of non-primed seeds under saline stress might be due to the excessive accumulation of both Na^+ and Cl^- in tissues and also by the osmotic and ionic stress caused by high Na^+ concentrations in soil (Ucarlı 2020) [32]. The increase in germination percentage of PA primed seeds might be due to the presence of organic compounds including organic acids and several derivates of alcohol (Grewal *et al.*, 2018) [14]. According to Nelson *et al.* (2009) [33] the active germination stimulant in PA is the presence of karrikinolide. PA priming might be helpful in minimizing salt-induced osmotic stress and improving water absorption in seedlings (Theerakulpisut *et al.*, 2017) [34].

The shoot and root lengths are the most important parameters for salinity stress because roots are in direct contact with the soil and absorb water while the shoot transports water to other plant components (Jamil and Rha 2004) [35]. In this study, the PA primed seeds showed gradual increase in root and shoot length when the concentration of dilution increased up to T_7 (1:500 PA). Maximum root length (17 cm) and shoot length (13.92 cm) was recorded by T_7 (1:500 PA), followed by T_6 (1:400 PA) which recorded 15.27 cm root length and 11.47 cm shoot length (Fig. 2). The presence of active compounds, such as growth hormones contributes to the enhanced seedling growth in PA primed seeds. Also, the seeds primed with PA reduced the harmful effects of salt stress in rice plants and it suppressed Na^+ uptake and stimulated K^+ accumulation in plant shoots (Jamil *et al.*, 2014) [36]. Seed soaking in solutions of PA can significantly enhance the content of abscisic acid and the antioxidant enzymatic activities in the roots and reduces the damage of salt stress to rice seedlings (Theerakulpisut *et al.*, 2017) [34].

The PA primed seeds had greater seedling dry weight than the control (0.129 mg 10 seedling^{-1}) and hydro primed seeds (0.155 mg 10 seedling^{-1}) under saline stress condition. The highest value of seedling dry weight was recorded in seeds primed with 1:500 PA (0.179 mg 10 seedling^{-1}) under saline stress condition followed by T_6 (0.170 mg 10 seedling^{-1}). Similarly, the VI I and VI II was higher in T_7 (1975 & 11.43) when compared to control (608 & 4.28). PA derived from plants is an effective stimulant that promotes seed germination and enhances seedling vigour (Rokich *et al.*, 2002) [37]. Ling *et al.* (2009) [38] evaluated the effect of PA on maize seed germination and seedling growth at different concentrations and found that PA had significant effects on germination rate, germination index, and vigour at 1:300 dilution. The acidity and the presence of butenolide in PA act as a germination stimulant and also increased seedling vigour and salt tolerance of rice seedlings (Staden *et al.*, 2000 & Daws *et al.*, 2008) [39, 40].

The investigation by Fackovcova *et al.* (2020) [41] showed that using pyroligneous acid had no harmful effects on non-target sensitive ecosystems and also the pyroligneous acid can be applied many times in order to increase its efficiency. Furthermore, treating the seeds with PA increases crop resistance.

Table 1: Effect of seed priming with PA on seed germination and seedling growth under saline stress condition

Treatment	Germination %	Root length (cm)	Shoot length (cm)	Dry matter production (mg 10 seedlings^{-1})	Vigour Index - I	Vigour Index - II
T_1	89 (70.63)	18.15	17.12	0.176	3143	15.72
T_2	41 (39.81)	10.28	8.20	0.129	608	4.28
T_3	51 (45.57)	13.28	9.10	0.155	1140	7.88
T_4	46 (42.13)	14.08	9.91	0.160	1100	7.34
T_5	50 (45.00)	14.63	10.67	0.168	1264	8.39
T_6	56 (48.44)	15.27	11.47	0.170	1495	9.51
T_7	64 (53.13)	17.00	13.92	0.179	1975	11.43
T_8	57 (49.02)	15.14	11.08	0.163	1494	9.23
Mean	56.75 (48.44)	14.73	11.43	0.162	1527	9.22
S.Ed	2.39	0.75	0.54	0.010	83.72	0.85
CD (P=0.05)	4.93	1.55	1.12	0.021	172.80	1.75

(T_1 – Absolute control (non-primed seeds without stress), T_2 – Control (non-primed seeds under stress), T_3 – Hydro priming, T_4 – Seed priming with 1:200 PA, T_5 – Seed priming with 1:300 PA, T_6 – Seed priming with 1:400 PA, T_7 – Seed priming with 1:500 PA, T_8 – Seed priming with 1:600 PA)

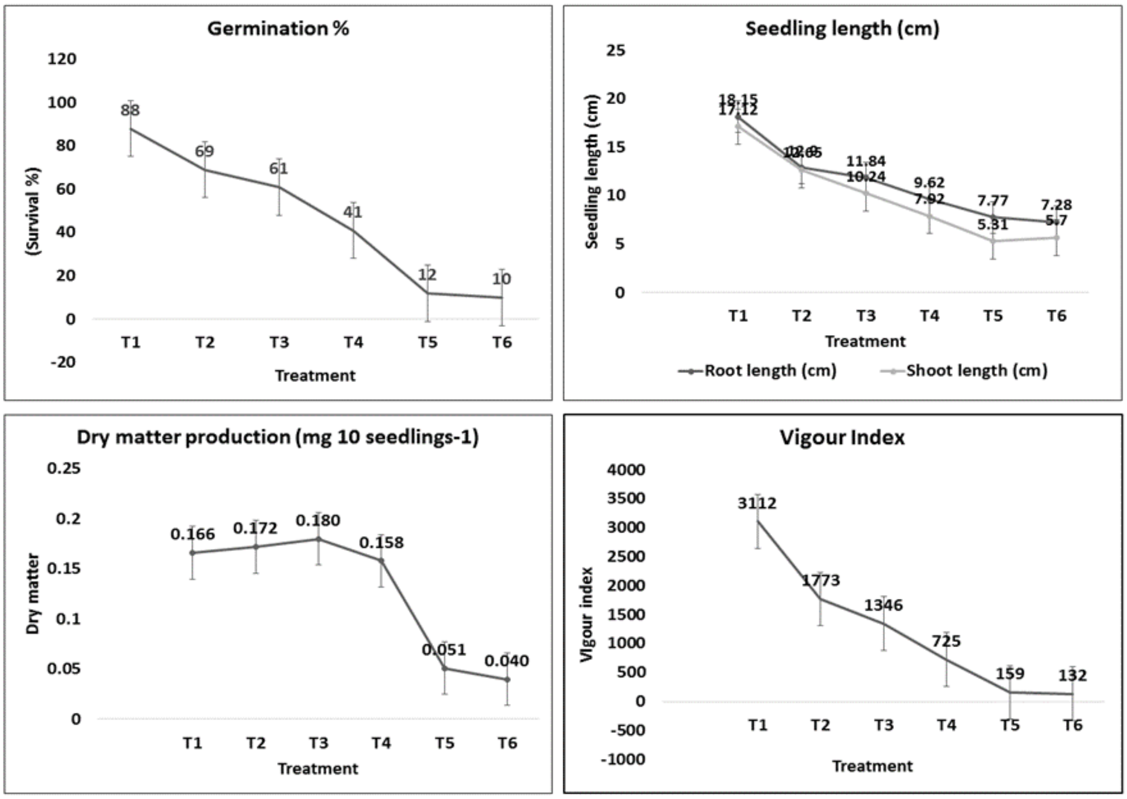

Fig 1: Effect of saline stress on seed germination and seedling growth (T_1 – Control, T_2 – 60mM NaCl, T_3 – 80mM NaCl, T_4 – 100mM NaCl, T_5 – 120mM NaCl, T_6 – 150mM NaCl)

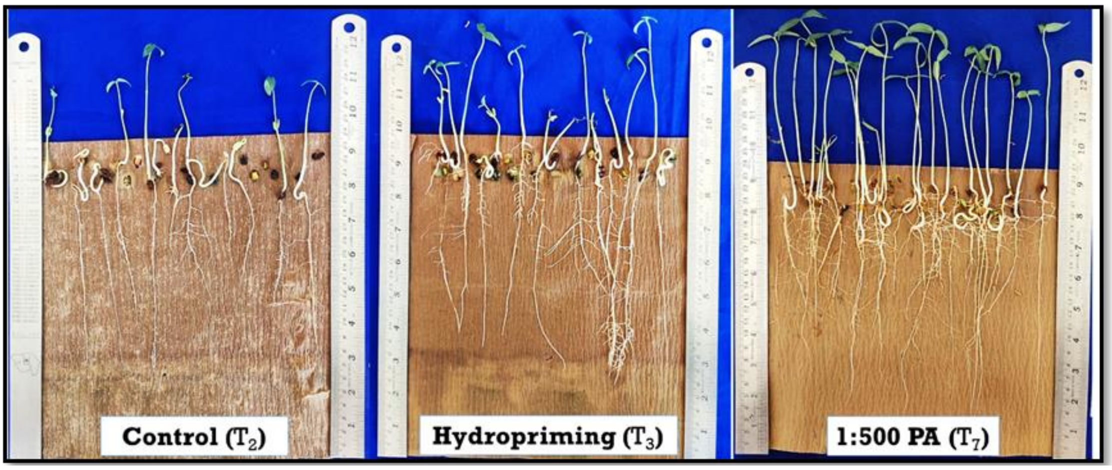

Fig 2: Effect of seed priming with PA on seedling growth in greengram under saline stress

Conclusion

The result of the present study clearly indicates that the seeds primed with 1:500 PA could alleviate the negative effects of saline stress in green gram seedling. Thus, seed priming with PA might be a feasible approach to promote greengram seed germination and crop growth under stress. However, the mechanisms of PA on seed germination and seedling growth under stress condition still deserves further research. The research might give a support for the further utilization of PA as a potential seed priming agent.

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