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Elucidating the Impact of Priming Substrates on Seedling Survival and Seed Quality of China Aster †

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Abstract: Germination or seed quality of China aster is a crucial feature affecting seedling survival and establishment whilst seeded directly in the field. Moreover, freak weather events in the changing climate scenario and biotic stress have often resulted in poor seedling quality and survival of China aster. Subsequently, to scrutinize the impact of a range of priming techniques on germination, seedling survival and growth of cv. Powderpuff of China aster newly introduced in Kashmir valley was undertaken at the Plant tissue Culture laboratory. Seeds were subjected to two treatment methods (3 hydro-priming and 2 halo-priming), constituting a total of six treatment combinations (P_0-P_5) in CRD (completely randomized design) with four replications. The analysed variables were seedling survival percentage, germination percentage, seedling collar diameter, seedling fresh weight, shoot/root ratio and the number of leaves/seedling. The analysed data on the influence of priming treatments on germination percentage is depicted that different priming agents are having a significant influence on pre and post-germination attributes. Significantly maximum germination percentage (87.50%), seedling survival percentage (81.95), seedling fresh weight (0.0031g), seedling collar diameter (0.101 cm), number of leaves seedling^{-1} (7.01) and shoot-root ratio (1.044) was recorded in treatment P_5 (2% KNO_3 18 h) and minimum (42.50) in case of control (P_0) i.e., un-primed seeds. Halo-conditioning with KNO_3 for 12h significantly improved, germination percentage, seedling survival percentage, seedling diameter, leaf number per seedling and shoot-root ratio. In conclusion, KNO_3 played a vital role in the establishment and survival of seedling in the field, under Kashmir conditions.

Keywords: China aster; growth; germination; priming; seed; seedling survival

1. Introduction

Callistephus chinensis (L.) Nees, more commonly known as China aster, belonging to family Asteraceae is one of the essential industrial flower crops. The genus Callistephus draws its name from two Greek words Kalistos' meaning 'most beautiful' and Stephos meaning 'a crown'. It is estimated to be grown in an area of 3500 ha in India [6].Amongst annual flowers, it ranks 3rd only after to Chrysanthemum and Marigold [19]. Its farming and cultivation have become trendy around larger cities for their versatile use as a loose and cut flower. The crop is used in making enchanting bouquets, buttonholes and garlands. In ornamental farming/gardening, it discovers its use as a cut flower, loose flower, bedding plant, pot plant and herbaceous border. Thus it has been extensively grown in South Asia and many other countries. Owing to the ever-increasing demand for quality seeds of Aster throughout India, there is a need to increase seed quality that would ensure good

returns for growers. In India, China aster seed available with nurseries is usually of poor quality which is an impediment for its wider cultivation.

Seed quality (germination, seedling stand, survival) is a decisive feature that decides the geological distribution and cultivation of any crop. China aster is highly sensitive to edaphic conditions like distribution of soil microbe inoculums especially fusarium resulting in poor germination, seedling survival, seedling stand and consequently results in poor quality seed and meagre returns. Furthermore, freak weather events in the changing climate scenario have often resulted in poor seed germination, seedling establishment and growth in India and elsewhere. Seed germination is a complex physiological course of action relating absorption of H_2O, metabolic breakdown of stored matter, seed respiration intended for energy metabolism, transcript (mRNA) production and mitochondrial refurbish and multiplication [3]. In recent years many tactics have been engaged to accelerate the speed of germination and to improve seedling uniformity. Pre-sowing treatment of seed priming has been proven as an effectual strategy for achieving rapid uniform emergence, as well as for recuperating seed vigour, viability, seedling stand even under the hostile situation. These methods can be classified as biological, chemical and physical. Numerous studies have accounted that priming (hydropriming, halopriming) reinforces the seeds to withstand various abiotic stresses during germination. the probable mechanisms stimulated by priming includes membrane repair due to accumulation of signalling proteins, epigenetic changes and enhanced antioxidant activity [13] starch metabolism under various conditions. A range of aspects such as cultivar, plant species, priming duration, priming substrate influences seeds response to priming treatment. However, reports regarding possible effects of seed priming on aster seed quality attributes and seedling survival/established are scant. Therefore, the identification of most effective priming agent is necessary to achieve the desired results such as improved germination, seedling survival, vigour. Consequently, the intent of the investigation was to probe the effects of seed priming on seed germination and seedling survival of China aster.

2. Material and Methods

2.1. Experimental Setup & Treatment Details

The experiment was conducted at the Plant Tissue Culture Laboratory, Division of FLA, SKUAST-Kashmir, Srinagar; J & K. The seeds of China aster were obtained from Department of Floriculture, Govt. of J&K. The seeds were surface sterilised and rinsed with distilled water Seeds were divided into 6 seed lots (3 hydro-priming and 2 halo-priming). The seeds were employed to 6 different priming treatments (T_0-T_5), and one untreated lot of seed (T_0). The study was evaluated as four replicates in a completely randomized design. The covered Petri plates were placed in an incubator at 25 ± 1 °C and kept at 16 h photoperiod. Seeds with noticeable radicle were considered as emerged. Data were recorded for 15 days on a daily basis for emergence with subsequent seedling assessment protocol as given in the handbook of the Association of Official Seed Analysts (AOSA1990). Ultimate germination percentage was calculated at the end of 15th day. Subsequently, four replications with 20 pre-germinated primed seeds were used as a basic sample and were sowed in propagations trays. 30 days after sowing various attributes viz, seedling survival percentage, seedling fresh weight, number of leaves per seedling, seedling collar diameter, and seedling shoot/root ratio was calculated empirically

2.2. Germination Percentage (G %)

Four replications with fifty seeds were kept in Petri plates covered with germination papers and then incubated in a seed germinator at 25 ± 1℃. The Petri plates were timely moisturized with distilled water in order to maintain optimum moisture level. The number of seeds germinated was counted daily for 15 days. A seed with visible radical protrusion was designated as germinated one. The number of germinated seeds was recorded daily, during the period of 15 days. A seed was considered germinated when its radicle emerged. Germination Percentage (G %) was calculated (ISTA, 1985) using the formulae as follows;

$$G\ \% = \frac{n}{N} \times 100$$

where
n = number germinated seeds
N = total number of seeds taken per lot

2.3. Seedling Survival %

Seedling survival percentage was calculated by setting the empirical formulae as shown below;

$$S.S\ \% = \frac{S}{g} \times 100$$

where,
S = number of seedlings survived after germination.
g = number of seedlings germinated.

The other apportioning attributes like Seedling fresh weight (SFW) was calculated after 30 days of seedling growth. 10 randomly selected seedlings were taken to calculate the fresh weights. After that, the mean fresh weight per seedling was deducted. Seedling collar diameter (cm) was analysed after 30 days of seedling survival. The observation was recorded with the aid of digital vernier calliper; the data was recorded in millimetres (mm) and later on converted to centimetres (cm). Number of leaves per seedling from each treatment, 5 seedlings were taken randomly and the number of leaves was calculated, 30 days after seedling growth. Ultimately seedling shoot/root ratio (S/R ratio) was deducted with Dry masses (mg) of shoot and root monitored separately from each treatment at random. The final shoot/root ratio was calculated using the equation as below,

$$S/R\ ratio = \frac{W_s}{W_r} \times 100$$

where,
W_s = dry mass of shoot (mg)
W_r = dry mass of root (mg)

2.4. Statistical Analysis

A completely randomised design (CRD) was employed in the experiments with four replications. Data analysis was carried out with the SAS programme (SAS Institute, Cary, NC, USA). Means were compared, by analysis of variance (ANOVA) at $p \leq 0.05$ level of significance and differences were divorced by Duncun's multiple range test.

3. Result and Discussion

Seed priming is a complex biochemical and physiological process modulated by cellular/solute osmotic potential, plant growth regulators, enzymatic activities [10,12]. Reports suggest that germination, seedling survival, the seedling stand of crop plants is restricted and limited by many factors that eventually reduce yield and quality. Hence it's important to stimulate attention towards the cost-effective strategies that improve crop growth and quality resulting in improved gross returns to the farming community.

Interestingly the results of our investigation (Figures 1 and 2) suggested that KNO_3 priming not only speed up seed germination rate but also considerably enhanced seedling survival, Timson germination index, seedling vigour index as indicated by longer radical lengths, hypocotyls lengths and shoot-root ratio. Timson germination index and germination percentage were enhanced by priming with H_2O and KNO_3 (Table 1). Germination percentage of primed seeds was higher with increased KNO_3 concentration and time of exposure. The extent of priming with H_2O and KNO_3 manipulated germination percentage differentially i.e., long-standing priming had an additional constructive outcome on germination. Treatment with 2% KNO_3 solution for 18 h resulted in a practically more acceptable germination percentage (85–87.50%). Therefore priming with 2% KNO_3

solution for a minimum of 12 and 18 h was deemed appropriate for increasing germination percentage. Even seeds subjected to H_2O priming for different durations showed more rapid germination (65% at 6 h, 70% at 12h and 77.50% at 18h) as compared to control (42.50%). Similarly, Timson germination index improved with increased priming duration (5.833 in 2 KNO_3 18 h, 5.667 in 2%KNO_3 12 h, 5.167 in H_2O 18 h, 4.667 in H_2O 12 h and 4.333 in H_2O 6 h) compared to control (2.833). Indicating that expanding the duration of priming treatment might result in a more positive influence on timson germination index. It's long been known that KNO_3 solution is an appropriate chemical approach for promoting germination in several plant species [14] improved germination percentage and Timson germination index KNO_3 treatment might be due to enhanced nitric oxide (NO) production in germinating seeds as a consequence of nitrite and nitrate decomposition [4,17]. The change may possibly be a retort to the interior alteration hastened by exposure to potassium nitrate (KNO_3). NO production is known to encourage the accessibility of nitrates and nitrites in germinating seeds and interacts with seed embryo photosynthesis [2,8]. Furthermore, KNO_3 is known to be engaged in endosperm putrefaction and augments the activity of amylase, protease which may perhaps have contributed to enhanced germination and other physiological indices [9]. Numerous studies have reported improved germination indices with KNO_3 treatment [4,18].

Our study was able to demonstrate an interesting event of significantly enhanced seedling survival percentage in China aster seedlings. Varied priming durations deferentially influenced seedling survival percentage. Improved seedling survival was recorded with extended priming duration and substrate concentration (81.94% in 2% KNO_3 18 h, 80.00% in 2% KNO_3 12 h, 64.24 in H_2O 18 h) as against 52.50% in case of control which was deemed as significantly lowest survival per cent. As noted previously priming reduces damage due to various abiotic and biotic factors [23] which contributes to better crop performance. Thus we assumed that KNO_3 priming might have improved the defence mechanism against a critical limiting factor i.e., fusarium wilt of seedling survival. Hence our findings suggest that KNO_3 priming could largely reduce the fungicide treatment on a seedling that contributes to significant input cost. This technique could be undertaken at a larger scale and possibly be a user and environment-friendly. No or few finding has been reported that suggest that KNO_3 priming to be an effective strategy to improve seedling survival. As noted earlier [5] KNO_3 priming improved seedling survival in *Digitalis purpurea*. We conducted a correlation analysis between the seedling survival percentage and other variables. The visualization of the correlation matrix between seedling survival percentage and other variables is illustrated in Figure 2. The matrixes symbolize positively high correlation between seedling survival percentage, seedling collar diameter, number of leaves per seedling etc.

Table 1. Effect of different osmotic agents and priming durations on germination percentage, seedling survival percentage and timson germination index.

Treatment	Germination Percentage	Seedling Survival Percentage	Timson Germination Index
T_0 (Control)	42.50 ± 17.08 (40.41 ^{c}) ^{x}	52.50 ± 5.00 (7.24 ^{c}) ^{y}	2.833 ^{b} ± 1.139
T_1 (Distilled water 06 h)	65.00 ± 25.17 (54.62 ^{bc})	55.16 ± 19.33 (7.34 ^{c})	4.333 ^{ba} ± 1.678
T_2 (Distilled water 12 h)	70.00 ± 16.33 (57.57 ^{bac})	61.03 ± 17.99 (7.75 ^{bc})	4.667 ^{a} ± 1.090
T_3 (Distilled water 18 h)	77.50 ± 12.58 (62.33 ^{ba})	64.24 ± 11.47 (7.99 ^{bac})	5.167 ^{a} ± 0.838
T_4 (KNO_3 2% 12 h)	85.00 ± 17.32 (71.01 ^{ba})	80.00 ± 8.06 (8.94 ^{ba})	5.667 ^{a} ± 1.156
T_5 (KNO_3 2% 18 h)	87.50 ± 18.93 (75.62 ^{a})	81.94 ± 12.78 (9.03 ^{a})	5.833 ^{a} ± 1.263

^{x} Data in parenthesis represent arcsine transformed. ^{y} Data in parenthesis represent square root transformed.

Table 2. Effect of different osmotic agents and priming durations on seedling fresh weight (g), seedling collar diameter (cm) and number of leaves/seedling.

Treatment	Seedling Fresh Weight (g)	Seedling Collar Diameter (cm)	No. of Leaves/Seedling
T_0 (Control)	0.018 ^{d} ± 0.004	0.068 ^{c} ± 0.006	3.756 ^{e} ± 0.008
T_1 (Distilled water 06 h)	0.026 ^{c} ± 0.004	0.083b ^{c} ± 0.016	5.756 ^{d} ± 0.008
T_2 (Distilled water 12 h)	0.027 ^{bc} ± 0.002	0.095b ^{a} ± 0.004	6.046 ^{c} ± 0.053
T_3 (Distilled water 18 h)	0.029 ^{bc} ± 0.004	0.086b ^{a} ± 0.002	6.256 ^{b} ± 0.008
T_4 (KNO_3 2% 12 h)	0.031 ^{ba} ± 0.003	0.099 ^{a} ± 0.013	7.014 ^{a} ± 0.008
T_5 (KNO_3 2% 18 h)	0.036 ^{a} ± 0.004	0.101 ^{a} ± 0.013	7.006 ^{a} ± 0.022

From the values of the number of leaves/seedling and seedling collar diameter, we deduced an interesting finding. The tendency of priming effect increased with prolonged duration and substrate concentration. The effectiveness of accelerated seedling collar diameter (0.101 ^{a} cm in 2% KNO_3 18 h and 0.086 ^{ba} cm in H_2O 18 h) and the number of leaves/seedling (7.014 in 2% KNO_3 18 h and 7.006 in 2% KNO_3 12 h) were observed in KNO_3 and H_2O priming compared to 0.068 cm and 3.756 respectively in control. It is noteworthy that the seedling raised from KNO_3 primed seeds despite having a maximum number of leaves/seedling resulted in lusher green seedlings depicting the improved chlorophyll content and vigour (Figure 1). These finding could be ascribed to the positive influence of K on the biochemical and physiological process of plant life like enhanced nitrate reductase (NR) activity [18,21]. NR is important in the creation of the antioxidant mechanism to forge ROS accountable for the volatility of photosynthetic complexes [16,20]. As noted and reported early KNO_3 substantially improves chlorophyll contents through cell expansion, osmoregulation and maintenance of cell membrane integrity

The results of our study suggested that halopriming (KNO_3) not only accelerated germination percentage, Timson germination index, seedling survival but also significantly enhanced seedling fresh weight, shoot-root ratio as indicated by longer radical lengths, shoot/root dry weight compared to control (Table 3). Seeds subjected to 2% KNO_3 priming for 18 h showed a marked build-up in seedling fresh weight (0.036 g), shoot dry weight (0.352 g), root dry weight and shoot-root ratio (1.044) as compared to control i.e., 0.018 g, 0.298 g and 0.572 respectively. It has been demonstrated in many crops that KNO_3 priming results in a significant increased physiological response [1].Plants raised from primed seeds are known to show structural amendments at all three levels (root, stem, and leaf) and the improved performance of plants is accredited to enhanced structural components cortical including vascular bundle thickness in leaf and increased pith cell area in stem [18]. Seeds primed in KNO_3 produced seedling having a maximum fresh weight per seedling in tomato [15]. It has been observed that the highest shoot fresh weight is produced with KNO_3 priming in safflower [11]. Similarly, numerous reports suggest that KNO_3 priming improves physiological aspects like fresh weight, dry weight, root/shoot lengths contrast to non-primed seeds [7,22].

Table 3. Effect of different osmotic agents and priming durations on shoot dry weight (g), root dry weight and shoot-root ratio.

Treatment	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot-Root Ratio
T_0 (Control)	0.298 ^{f} ± 0.001	0.289 ^{e} ± 0.005	0.572 ^{c} ± 0.005
T_1 (Distilled water 06 h)	0.299 ^{e} ± 0.000	0.317 ^{d} ± 0.005	1.036 ^{a} ± 0.017
T_2 (Distilled water 12 h)	0.325 ^{d} ± 0.000	0.328 ^{c} ± 0.005	1.027 ^{a} ± 0.015
T_3 (Distilled water 18 h)	0.328 ^{c} ± 0.001	0.332 ^{cb} ± 0.005	1.000 ^{b} ± 0.014
T_4 (KNO_3 2% 12 h)	0.333 ^{b} ± 0.001	0.337 ^{b} ± 0.005	1.001 ^{b} ± 0.014
T_5 (KNO_3 2% 18 h)	0.352 ^{a} ± 0.000	0.520 ^{a} ± 0.005	1.044 ^{a} ± 0.015

(**a**)

(**b**)

Figure 1. (**a**) Evaluation of seedling in propagation trays note vivid brighter green colours indicates an improvement in leaf chlorophyll content, (**b**) Visual differences of various conditioning agents on seedling growth. A sturdier seedling growth with KNO3 may be the main reason for enhanced seedling survival.

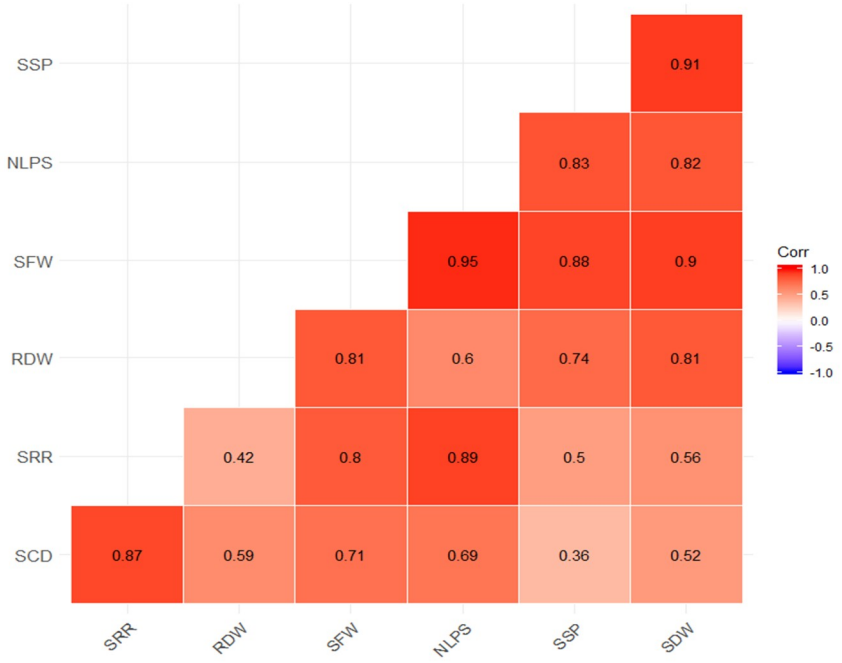

Figure 2. Visualization of correlation matrix between seedling survival percentage (SSP) vs. number of leaves per seedling (NLPS), seedling fresh weight (SFW), root dry weight (RDW), shoot-root ratio (SRR) and seedling collar diameter (SCD).

4. Conclusions

In summary, seeds primed with KNO_3 not only accelerated germination percentage, Timson germination index, the number of leaves/seedling, seedling collar diameter, dry matter but also significantly enhanced seedling survival percentage compared with those of control. In practice, it is recommended that China aster 'Powderpuff' seeds can be treated in 2% KNO_3 solution for 18h to obtain optimum seed germination and seedling rate/survival for favourable establishment in the field. Moreover, our findings advocate that KNO_3 priming can be used as a cost effective strategy to alleviate seedling survival at a larger scale. For farmers/growers perspective this technique could be promising to replace the obnoxious activity of pesticide application for improving seedling survival with no tangible ill effects on soil, seeds or on human health.

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Conflicts of Interest: Authors declare that they have no conflict of interest.

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