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Nutri and Vitamin priming methods improves carrot seed germination under salinity stress

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Abstract
Carrot was rated as salt sensitive crop, root yield declines by 14% for every unit increase in salinity (EC) beyond threshold of 1.0 dSm^{-1}. In the laboratory studies, Nutri-priming followed by vitamin priming had shown significantly higher performances than unprimed seeds in alleviation of salinity stress. Nutri-priming over unprimed seeds significantly increased Germination percentage, Seedling vigour Index-I, Seedling vigour Index-II, early emergence by 2.4 days and 52% reduction in Seedling electrolyte leakage. In all the treatments, progressive decline in seed quality parameters studied with storage was observed. Different intervals of primed seeds storage suggested that up to 30-days the primed seeds retain viability and vigour, thereafter significant decline at 60- and 90-days storage intervals.

Keywords: Carrot, priming, salinity

Introduction
Salinity is one of the major abiotic stresses that affect crop production in arid and semi- arid tropics. In hot and dry environments, high evapo-transpiration results in salt accumulation around the top 5.0 cm layer of the soil and thus this layer is more saline than the lower profiles. Seed sowing is usually confined to the top 2.5 cm soil that impedes successful crop production in sensitive crops. Seed germination and seedling growth of crops are the most sensitive stages of salinity. Priming is pre-sowing treatment given to seeds that activates the physiological state of the embryo that enables rapid and synchronous seeds germination. Priming allows some of the metabolic processes necessary for germination to occur without radical emergence. Insufficient seedling emergence and inappropriate stand establishment are the major constraints often faced by farmers in these areas that need attention.

Numerous studies reported on different vegetable crops suggested that seeds priming could improve the overall crop performance under salinity stress. Several vegetable seeds including carrots showed slow and erratic germination especially under adverse conditions of soil salinity. Carrot was rated as salt sensitive crop, root yield declines by 14% for every unit increase in salinity

(EC) beyond threshold of 1.0 dS m^{-1} (Mass,1986) [12]. Seeds of umbelliferae are noted for their dormancy and poor germination due to rudimentary embryos and embryo less seeds (Dale HM and Harrison PJ, 1996) [7]. Floral feeding insects such as Lygus bugs on developing seeds, while feeding inject toxic compounds from oral secretions were reported to be primary causes of embryo less seeds.

Presence of rudimentary embryos are often the cause for delayed germination in carrots (Robison RW 1954) [14]. Very little work has been conducted on carrot seeds, effects of different seeds priming methods on germination, vigour, seedling establishment and field performance in saline soils.

Materials and Methods
The laboratory research studies were conducted in the Department of Crop Physiology laboratory, College of Horticulture, Mojerla, Telangana during 2020-21. The experiment was laid out in completely randomized design (CRD) with four replications and seven treatments. The primed seeds were tested for storability at intervals of 7, 30, 60 and 90 days after seeds priming.

Laboratory study was conducted in petri dishes with primed seeds placed on top of two layers of blotter papers and incubated in the seed germinator at 25 ± 2 °C temperature and 90 ± 5 per cent RH. (ISTA, 1999) [8].

For each treatment 50 seeds were used and arranged in germinator in completely randomize design.

Data collection
Germination was considered when radicle protrusion was 1cm long. The seedlings with short, thick, and stunted roots were considered abnormally germinated. Germination percentage calculated every day until 14 days.

$$\text{Seeds Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total no of seeds}} \text{ x } 100$$

Mean germination time (days)
Mean Germination time was calculated based on the following equation of Ellis and Roberts (1981).

$$\text{Mean Germination Time} = \frac{\sum nt}{\sum n}$$

Where,
$\sum n = n_1 + n_2 + n_3 + n_4 + n_5 + \ldots\ldots\ldots\ldots\ldots + n_t$

n = number of seeds which were germinated on day t and t is the number of days t = t^{th} day counted from the beginning of germination test.

Seedling vigour index
For determination of seedling vigour, five normal seedlings were selected randomly from each replication and treatment at the end of germination test. The seedling vigour index was calculated by formulae suggested by Abdul Baki and Anderson (1973) [1].

Seedling vigour index I = Germination (%) x Seedling length (cm)

Seedling vigour index II = Germination (%) x Seedling fresh weight (mg)

Seedling Electrolyte leakage
Seedling Electrolyte leakage was calculated to know the membrane integrity of seedlings against soil salinity. Five normal seedlings were selected randomly from each treatment at the end of germination test (14 days) and these seedlings were placed in small beakers with 15ml double distilled water for 2 hour (readings were taken at hourly intervals) and these beakers were placed under EC meter for determining readings and expressed as seedling Electrolyte leakage in µ Siemens g^{-1} cm^{-1}.

Germination value
Germination value is obtained by combining both speed and completeness of germination into a composite score as described by Czabator (1962) [5].

Germination Value = MDG × PV

Where
MDG= Mean daily germination
PV= Peak value or largest quotient obtained when all of the cumulative germination percentages were divided by the respective time interval.

Data analyses
Pooled data obtained different seed testing intervals were subjected to repeated measures analyses procedure with NCSS 12 Statistical Software (2018). The 2-factor mixed model procedure was used with treatments, primed seeds storage intervals as fixed effects and replicates as random. Whenever ANOVA indicated significant differences, treatment means were compared using Tukey's HSD test at *p* =0.05

Results and Discussion
Germination Percentage
Maximum germination percentage (79.21%) was observed in T_5 (Nutri-priming)
and minimum germination percentage was recorded in Non primed seeds. Seed priming stimulates the activities of enzymes like α-amylase which then accelerate the breakdown of food reserves and supply of energy to growing embryos (Kaur *et al*., 2002) [10]. Seeds priming improves crop performance by triggering physiological, molecular, and biochemical changes (Chen *et al*., 2012). The result is in conformity with the findings of Sajjan *et al*. (2017) [15] who observed that the seeds priming enhanced the germination rate, better allometric attributes, faster emergence of seedlings as well as early germination.

Mean Germination Time
Mean germination time is the reciprocal of the rate of germination. Among all treatments T_1(non-primed seeds) recorded highest mean germination time (8.78 days) and lowest mean germination (6.30 days) was observed in T_7 (Vitamin Priming). Ascorbic acid pretreatment of seeds counteracted the decrease in ascorbate oxidase (AO) induced by salt stress but appeared to act synergistically with salt stress to decrease proline dehydrogenase. The application of ascorbic acid to carrot seeds apparently increased antioxidant activity, leading to an increase in resistance to salt stress. The above findings were accordance with Khan *et al*. (2011) [11].

Seedling Vigour Index
Maximum seedling vigour index I and II (675.21&15.26) was found in Nutri-priming and minimum values (343.10 & 8.12) was recorded in non-primed seeds. Higher vigour index recorded by nutri-priming might be due to better crop growth as evident from data on shoot length, root length and flesh weight. Ozturk *et al*. (2006) [13] reported that Zn is involved in the vital physiological process in the early stage of radicle and coleoptile during seeds germination.

Seedling Electrolyte Leakage
Maximum electrolyte leakage (183.61 $\mu Sg^{-1}cm^{-1}$) was recorded in non-primed seed and minimum leakage (120.70 $\mu Sg^{-1}cm^{-1}$) was observed in nutri-priming. Our results showed that salinity stress resulted in higher electrolyte leakage, but the increase in electrolyte leakage was greater under non-primed seeds as against primed seeds. According to J. FU and B. Huang (2001) [6], cell membrane stability is affected by lipid peroxidation due by active oxygen species under various stress conditions. Priming with ascorbic, $ZnSO_4$ and $MnSO_4$ improved the drought resistance, determining proline accumulation and antioxidant action of ascorbic acid and phenolics. The specific activity of these chemicals allows maintaining a proper water content in tissues, provide structural stability and functional membranes and

consequently a more uniform and vigorous growth of seedlings.

Germination Value

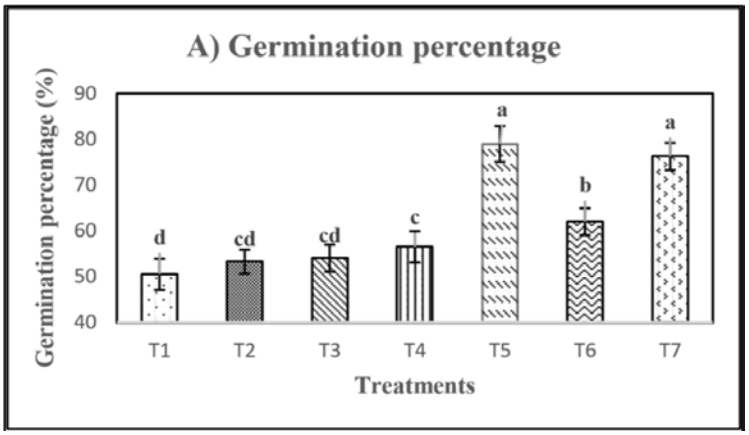
Germination value is obtained by combining both speed and completeness of
germination into a composite score. Maximum germination value (58.73) was observed in nutri-priming and minimum germination value (17.90) recorded in non-primed seeds. Faster emergence of primed seeds might be due to metabolic repair during imbibition (Bray *et al.*, 1989) [3] and build of germination enhancing metabolites (Basra *et al.*, 1995), while higher and synchronized emergence was the consequence of reduced physiological non-uniformity in the seeds due to priming (Juraimi *et al.*, 2012) [9]. Nutri-priming recorded higher germination value than non-primed seed.

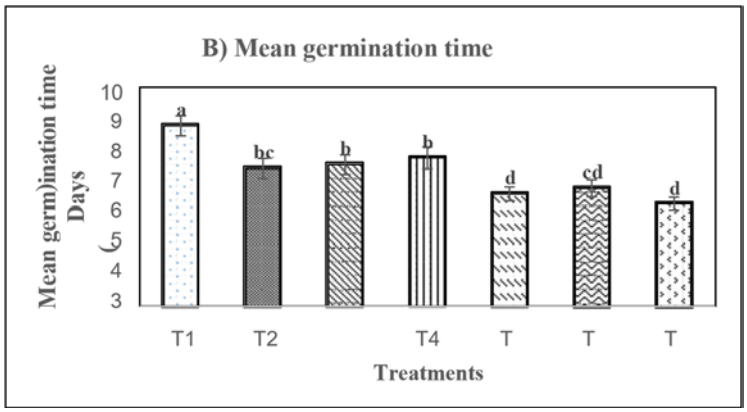
Table 1: Effect of different seed priming methods and storage intervals in alleviating the salinity stress under laboratory conditions

Fixed Effects	GP (%)	MGT (days)	SV-I	SV-II	SEL (µ Siemens g−1 cm−1)	GV
Treatments (T)						
T_1: Non-Primed seeds	50.50 ^{d}	8.78 ^{a}	343.10 ^{g}	8.12 ^{d}	183.61 ^{a}	17.90 ^{d}
T_2: Hydro priming	53.00 ^{cd}	7.41 bc	371.80 ^{f}	9.64 ^{c}	161.20 ^{b}	33.20 ^{ac}
T_4: Halo priming	54.01 ^{cd}	7.53 ^{b}	405.90 ^{d}	10.43 ^{bc}	151.12 ^{c}	33.11 ^{ac}
T_4: Osmo priming	56.55^{c}	7.75 ^{b}	394.70 ^{e}	10.18 ^{c}	137.73 ^{d}	21.60 ^{d}
T_5: Nutri-priming	79.21 ^{a}	6.6 d	675.21 ^{a}	15.26 ^{a}	120.70 ^{f}	58.73 ^{a}
T_6: Hormonal priming	62.01 ^{b}	6.78 cd	452.52 ^{c}	11.71 ^{b}	126.5 1^{ef}	36.9 1^{c}
T_7: Vitamin priming	76.25 ^{a}	6.30 ^{d}	611.53 ^{b}	14.76 ^{a}	131.83 ^{de}	46.80 ^{b}
Storage Intervals (S)						
S1: 7 DAP	65.85 ^{a}	6.97 ^{d}	518.00 ^{a}	12.72 ^{a}	142.01 ^{bc}	38.20 ^{a}
S2: 30 DAP	62 ab	7.22 ^{c}	474.01 ^{b}	11.74 ^{ab}	143.11 ^{b}	35.81 ^{b}
S3:60 DAP	60.2 ^{b}	7.40 ^{b}	447.02 ^{c}	11.01 ^{b}	145.01 ^{a}	34.60 ^{c}
S4: 90DAP	58.2 ^{c}	7.64^{a}	413.00 ^{d}	10.31 ^{c}	147.00 ^{a}	33.31 ^{d}

(Values followed by different letters in a column were significantly different; Tukey's Honest Significant Different (HSD) test at $P \leq 0.05$).

DAP: Days after Priming (GP: Germination Percentage; MGT: Mean Germination Time; GI: Germination Index; SVI-I: Seedling
Vigour Index-I; SVI-II Seedling Vigour Index-II; SEL: Seedling Electrolyte Leakage; GV: Germination Value)





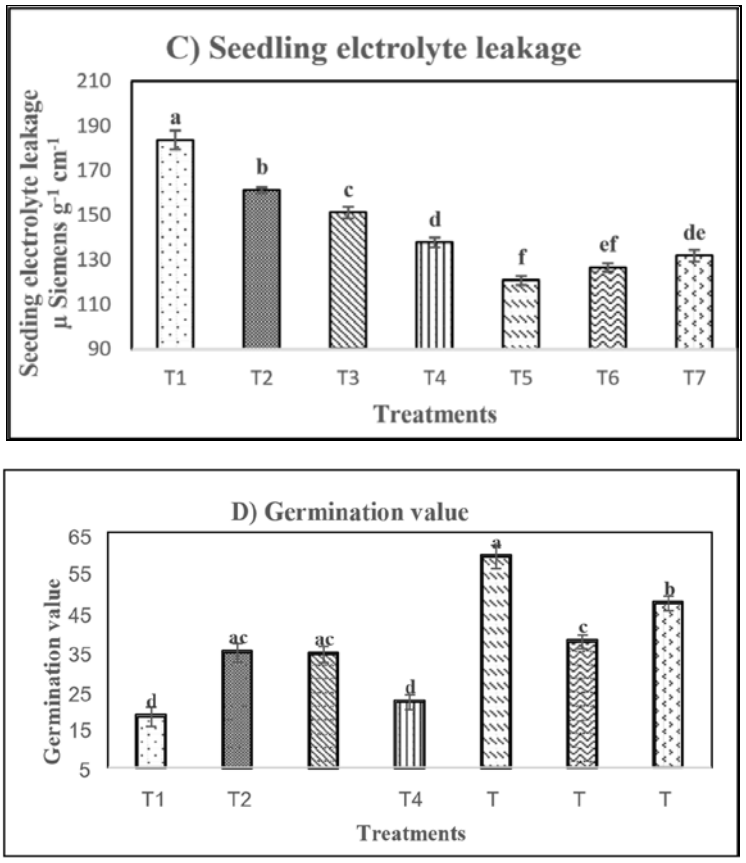


Fig 1: Effect of different priming treatments on A) Germination percentage B) Mean germination time C) Seedling electrolyte leakage D) Germination Value. Pooled data of 7,30,60 and 90 DAP under laboratory conditions. Error bar represent SEM and bars with different letters significantly different by Tukey's HSD test at $P \leq 0.01$. T_1: Non-primed seeds; T_2: Hydro priming; T_3: Halo priming; T_4: Osmo priming; T_5: Nutri-priming; T_6: Hormonal priming; T_7: Vitamin priming

Conclusion

Nutri-priming and vitamin priming are promising methods for carrot seed under saline conditions. Nutri-priming recorded the highest in all aspects and closely followed by vitamin priming. 7 days and one month after priming performed better than the remaining storage intervals i.e., 60 and 90 days after priming. Progressive deterioration of primed seed was observed at 60 and 90 days after priming.

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