EFFECT OF NACL PRIMING DURATION AND CONCENTRATION ON GERMINATION BEHAVIOR OF FENUGREEK

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Abstract:

Priming is one of the seed enhancement methods that might be resulted in increasing seed germination under stress conditions such as salinity, temperature and drought stress. The objective of this study was to develop an optimal priming protocol for fenugreek (*Trigonella foenumgraecum* L.) and determinate the effect of NaCl seed priming on seed germination. Fenugreek seeds were primed with four concentrations of NaCl as priming media (0, 4, 6 and 8 g L^{-1}) for 12, 24 and 36 hours. Results indicated that different priming concentrations and duration have significant effects on total germination percentage, mean germination time, germination index and coefficient of velocity of fenugreek seeds and the best result was obtained with (4 g L^{-1}, 36h). The result of this experiment showed that under undesirable conditions such as salinity stress, priming with NaCl (4g L^{-1}, 36 h) can prepare a suitable metabolic reaction in seeds and can improve seed germination.

Keywords: Fenugreek, germination, priming duration, priming concentration.

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a flowering annual plant, with autogamous flowers. This crop is native to an area extending from Iran to northern India and widely cultivated in the world [1]. The production of this crop is affected by environmental stress such as: drought, salinity, chilling and heat [2]. In fact, salinity is the major abiotic stress and significant factor affecting crop production all over the world and especially in arid and semi-arid region [3]. That's why, The need to develop crops with higher salt tolerance has increased within the last decade. Seed priming is one of the physiological methods, which improves seeds performance for many crops, particularly seeds of vegetables and small seeded grasses [4] [5] and it is defined as a pre-sowing treatments in water or in an osmotic solution that allows seed to imbibe water to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat [6], [7], [8]. Examples of such osmotica are $CaCl_2$, NaCl, KCl, PEG and KNO_3 etc… [9]. For years, a lot of work has been carried to improve the germination rate and uniformity of growth and to reduce the emergence time of many vegetables and field crops [10]. In fact, rapid and uniform field emergence is essential to achieve high yield with having good quality and quantity in annual crops [11]; therefore, seed priming is used for improvement of germination speed, germination vigor, seedling establishment and yield [12]. The technique of seed priming is becoming familiar to farmers in the world, and has been promoted there on a range of crops, for example wheat [13], maize [14], and mung bean [15]. There are reports that seed priming permits early DNA replication, increase RNA and protein synthesis, enhances embryo growth, repairs deteriorated seed parts and reduces leakage of metabolites [16]. NaCl priming could be used as useful method for improving salt tolerance of seeds. Previous studies on tomato [17] and melon [18] showed that seed priming improves seed germination, seedling emergence and growth under saline conditions. Improvement in priming is affected by some factors such as plant species, water potential form priming factor, priming duration, temperature, vigor and seed primed storage condition [19]. So, optimization of priming technique is very important to achieve the best time and concentration combination. That's why; the objective of this study was to evaluate the effect of NaCl seed priming with different concentration and duration on fenugreek germination behavior.

2. Material and Methods

2.1. Seed material

Seed material is composed of fenugreek seeds (*Trigonella foenumgraecum* L.,) that are commonly cultivated in Tunisia and used in the present study. Seeds were initially treated with a 5.0 % solution of sodium hypochlorite for 3 min for surface sterilization and then they were rinsed 3 times with distilled water for 2 min.

2.2. Priming techniques

The study was conducted in the laboratory of the Department of Agronomy and crop science of the High Institute of Agriculture Chott Mariem, Tunisia to determine seed priming effects on germination, and seedling growth of a Tunisian fenugreek cultivar. Seeds were primed with four levels of NaCl as priming media (0, 4, 6, 8 g L^{-1}) for 12, 24 and 36 hours at 25°C. Experimental units were arranged factorial in a completely randomized design with three factors which are priming media (NaCl, and untreated seed (control)), priming concentration (4, 6, and 8 g L^{-1}) and priming duration (12, 24 and 36 h) in a completely randomized design with five replications and 30 seeds per replicate. Dry fenugreek seeds were considered as a control treatment (none primed). After treatment, seeds were given three surface washings with distilled water and re-dried to original weight at the laboratory temperature. Seeds from each of the treatments were placed in 90-mm-diameter Petri dishes on between two layers Watman filter paper and then moistened with 10 ml of distilled water. Seed was kept at room temperature (25°C) under normal light. The number of germinated seeds was counted daily for 7 days after which no further seed germination was occurred. The appearance of 2 mm or more of radicle was considered as germination. Parameters measured in this experiment are given below:

Total germination (TG) measured in the seventh day using the formula GT (%) = (total number of germinated seeds/ total seed) x 100.

Mean germination time (MGT) was calculated according to the equation MGT = ΣDn/Σn [20]. Where (n) is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination.

The germination Index (GI) was calculated as described in the Association of Official Seed Analyst [21] by following formula: GI = Σ (TiNi). Ti: number of day after sowing and Ni: number of germinated seeds in the day.

The coefficient of velocity (CV) was calculated using the following formula:

C V= 100 [∑Ni / ∑Ni Ti], [22].

2.3. Statistical analysis

All the data were subjected to an analysis of variance, using SPSS 13.0 software and the difference between means were compared by Duncan multiple range test at 5% level of probability.

3. Results and Discussion

Results indicated that studied traits were affected by the experimental factors and there was completely significant difference between control (non primed seeds) and primed seeds (Table 1, 2). Total germination percentage (TGP) increased with increase in seed priming duration compared to control (dry seed). Higher TGP was obtained at 36 h seed priming duration (82.33%) followed by seed primed for 24 h (71.83%). Greater TGP was obtained for seed treated with 4 g L^{-1} NaCl (89.55%) followed by 6 g/l NaCl (80.66%). Effects of D, C for TGP were significant but the D x C interaction was not significant for TGP. Primed seeds recorded higher TGP as compared with non primed seed (Table 1). Variance analysis and mean comparison results displayed that mean germination time (MGT) was affected by different priming concentration and seed priming duration.

The least MGT was obtained from 4 g L^{-1} priming concentration (4.01) and 36 h NaCl priming duration (4.29) treatments. Less MGT was attained from NaCl seed priming treatment than control (Tables 2). Effects of D, C for MGT were significant and the D x C interaction was significant too for MGT (Table 1).

Variance analysis and mean comparison results displayed that germination index was affected by different priming concentration and seed priming duration. The highest germination index was attained for 36 h priming duration and 4 g L^{-1} concentration of priming (103.30 and 108.80 respectively). But generally, Primed seed recorded higher germination index as compared with non primed seed. Effects of D, C for germination index were significant but the D x C interaction was not significant (Table 1).

Priming duration and concentration significantly affected the coefficient of velocity with highest values observed in seeds primed with 4 g L^{-1} NaCl (28.83) for 36 h (25.58) and Primed seeds recorded higher coefficient of velocity values than non primed seed (Table 2). Effects of D, C for germination index were significant but the D x C interaction was not significant.

Priming the seeds for 36 hours at 4 g L^{-1} NaCl had better effects on Total germination percentage, mean germination time, germination index and coefficient of velocity compared with other treatments (Table 2).

Table 1. Variance analysis of studied traits in fenugreek.

		Mean square			
Source of Variation	**df**	**Total Germination**	**Mean Germination Time (MGT)**	**Germination Index (GI)**	**Coefficient of Velocity (CV)**
Duration (D)	*2*	*870.338* *	*1.847* *	*713.317* *	*145.907* *
Concentration (C)	*3*	*4964.954* *	*5.581* *	*1945.089* *	*504.590* *
D x C	*6*	*77.875* ns	*0.588**	*186.406* ns	*34.194* ns
Error	*48*	*71.214*	*0.146*	*156.633*	*33.531*
Total	*60*				

(*) Significant at the 5% levels of probability according to Duncan test; (ns) Non significant

Table 2. Means comparison of studied traits in fenugreek by Duncan multiple range test

		Total germination (%)	**Mean Germination Time (days)**	**Germination Index**	**Coefficient of velocity**
Duration (hour)	12	70.16[b]	4.77[a]	92.45[b]	20.86[b]
	24	71.83[b]	4.85[a]	93.55[b]	20.94[b]
	36	82.33[a]	4.29[b]	103.30[a]	25.58 [a]
Control (dry seed)		68	4.96	86	18.8
Concentration (g/l)	0	80.55[b]	4.54[b]	96.80[b]	22.36 [b]
	4	89.55[a]	4.01[c]	108.80[a]	28.83 [a]
	6	80.66[b]	4.53[b]	98.86[b]	23.83 [b]
	8	48.21[c]	5.48[a]	81.26[c]	14.81 [c]
Control (dry seed)		68	4.96	86	18.8

Means followed by the same letters are not significantly different according to Duncan test at 5% level.

4. Discussion

In the present study, primed seeds, with 4 g L^{-1} for 32 h, clearly improved germination by increasing germination rate and uniformity, and reducing mean germination time compared to untreated seeds. This may be related to rapid water uptake comparing to the control treatment. Similarly, Kaya et al. [23] reported that primed seeds had more rapid water uptake abilities than untreated seeds in sunflower. Priming may improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells [23]. Bewley and Black [24] reported that priming allows the hydration of membranes and proteins, and the initiation of various metabolic systems. These are arrested when the seeds are dried or moisture is withheld, but recommence when the seeds imbibe water for the second time. This positive response of seed priming is consistent with the findings of Coolbear and Grierson [25] who reported that higher germination rate was a result of higher levels of nucleic acid in primed seeds of tomato cultivars. They indicated that increase in nucleic acid content in primed seeds was due to an enhanced ribonucleic acid (RNA) synthesis during and after priming treatment. According to Gray et al. [26] seed priming modifies the embryonic axis growth and consequently seedling development.

Under all concentrations and priming durations, mean germination time was lower in primed seeds but optimal time of priming for fenugreek seeds was 36 hours in the solution of NaCl (4 g L^{-1}). A shorter time of priming seems to have little effect on the seed parameters of fenugreek. These results are in accordance with the observations of Jeong et al. [27] who demonstrated that priming with PEG 8000 (-0.5 MPa) lowered the mean germination time of seeds of lettuce, carrot and onion. Goobkin [28] and Ozbingol [29] also reported that PEG 6000 solution –treated tomato seeds germinate faster than untreated seeds and this is due to more rapid water uptake.

The probable reason for early emergence of the primed seed maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed [30]. Yamauchi and Winn [31] indicated that seed priming may help in dormancy breakdown possibly by embryo development and leaching of emergence inhibitors which resulted in an earlier start of emergence. Rapid seedling establishment might also minimize crop risk due to

environmental conditions or insect and disease problems during field emergence [32].

Germination index is a good indicator of seed vigor. That's why; higher value of germination index will indicate the vigor of seeds. Significant improvement in germination index might be the result of early and synchronized seed. It might also be the result of better development of genetic repair mechanisms during the course of priming operations leading to improved germination index [33]. Ruan et al. [34] has demonstrated that KCl and CaCl2 seed priming had improved germination index of rice. Improving germination and coefficient of velocity in treated fenugreek seeds may be explained by an increase of cell division in the seeds [35].

Better results of NaCl priming (4g L^{-1} for 36 h) treatment could be due to the uptake of Na+ and Cl- ions by the seed, maintaining a water potential gradient allowing water during seed germination [36]. Thus, this protocol (4g L^{-1} for 36 h) is considered as the optimal priming protocol for fenugreek germination tests.

5. Conclusions

The results of experiment showed that priming with NaCl improves germination indices. Furthermore, the germination of priming seeds was begun earlier than control seeds, and subsequently these seeds will establish more quick under salinity stress. This method is simple, cheap and it does not require any special equipment, so farmers can use it to increase percent and homogeneity of emergence of plants under environmental stresses.

Further, this study needs to investigate the effects of priming on later growth and development stages of this plant.

6. References

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