

# Hydropriming and NaCl influences on seedling growth in Fenugreek (*Trigonella Foenum-Graecum*).

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## Introduction

Fenugreek, which has been produced for ages, is one of the most favorite fodder and medicinal plants of the Eastern Mediterranean and subtropical climate countries. It was a widespread field crop in ancient Egypt and in the Roman and Greek empires. It is native at the coastal regions of the Mediterranean, but India, China, Egypt, Turkey and Morocco are also counted as production areas. Due to its excellent adaptation abilities this plant can be produced as spring sown plant in various countries of temperate climate [40]. The seed of Fenugreek contains alkaloid, Colin, bitter material, fatty acid, protein and vitamin C. It also contains diastase, alkaloids, and materials, which are excellent remedies for dysorexia and weakness resulting from emaciation. Water that is saline contains significant amounts of dissolved salts. In this case, the concentration is the amount of salt in water, as expressed in ppm. If water has a concentration of 10,000 ppm of dissolved salts, then one percent of the weight of the water comes from dissolved salts [68]. Salinity is a major environmental constraint to crop productivity throughout the arid and semi-arid regions of the world [18].

Salinity has reached a level of 19.5% of all irrigated-land (230 million ha of irrigated land, 45 million ha are salt-affected soils) and 2.1% of dryland (1500 million ha of dryland agriculture, 32 million are salt-affected soils) agriculture worldwide. According to the FAO, around 1.5 million ha of land in Turkey have both salinity and sodicity problems [16]. Seed germination is a major factor limiting the establishment of plants under saline conditions. Salinity may cause significant reductions in the rate and percentage of germination, which in turn may lead to uneven stand establishment and reduced crop yields [18].

Salt tolerance at germination stage is an important factor, where soil salinity is mostly dominated at surface layer. High concentration of salts have detrimental effects on germination of seeds [35,51,57]. Plant growth is ultimately reduced by salinity stress but plant species differ in their sensitivity or tolerance to salts [65,48,53]. Generally, salt stress causes both osmotic stress and ionic stress [67]. There are many strategies to overcome the negative effects of drought and salinity. A good strategy is the selection of cultivars and species for salinity and drought conditions [5]. The plant is relatively salt resistant and has no much needs of soil fertility [46]. But in production of medicinal plants, seed germination is a very important problem. The seeds are occasionally sown in seedbeds having unfavorable moisture because of the lack of rainfall at sowing time [62], which results in poor and unsynchronized seedling emergence [60]. A major constraint to seed germination is soil salinity, a common problem in irrigated areas of Iran, with low rainfall [44].

Soil salinity may affect the germination of seeds either by creating an osmotic potential

external to the seed preventing water uptake, or through the toxic effects of Na<sup>+</sup> and [Cl<sup>sup.-</sup>] ions on the germinating seed [45]. Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment [42]. Under these stresses there is a decrease in water uptake during imbibitions and furthermore salt stress may cause excessive uptake of ions [12]. Seed germination is mostly an issue in medicinal plant seeds emergence [50]. Good seedling establishment is an important constraint to such crop production [27]. Poor seedbed, low quality seed, environmental stresses such as high and low temperature and salinity constrains to good establishment include [66]. A robust seedling establishment enhances competitiveness against weeds, improves tolerance to environmental stresses and maximizes biological and grain yields [22]. Seed priming is a pre-sowing strategy for improving seedling establishment by modulating pregermination metabolic activity prior to emergence of the radicle and generally enhances germination rate and plant performance [11,64,21]. Seed priming is an efficient method for increasing of seed vigor and improvement of germination and seedling growth (Ascherman-Koch et al., 1992, 31). Priming allows seed hydration to initiate the early events of germination, but not permit radicle emergence, followed by drying to initial moisture [43,6]. 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 [43]. Seed priming is seen as a viable technology to enhance rapid and uniform emergence, high vigour and better yields mostly in vegetable and flower species [15,10]. Hydrated seeds with higher germination percentage under salt stress or micronutrient application increased tolerance of seeds to salt stress. In addition, reported protocol is simple, cheap and does not require expensive chemicals and sophisticated equipment. The protocol has practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions. The objective of this study was to explore the effects of irrigation by saline water on seedling vigour in Fenugreek (*Trigonella Foenum-Graecum*) Seed's.

## Materials and methods

In order to determine the effect of hydropriming on germination in fenugreek seed's, under irrigated by saline water, an experiment was conducted in 2011 at Laboratory Sciences, Islamic Azad University Shahr-e Qods Branch by a completely randomized design with three replications and the first, seed viability was determined by Tetrazolium test method.

After disinfecting, seeds were put in disinfected Petry dish. Each Petry dish contained 100 seeds. Three replicates of 100 seeds were put between double layered rolled. The rolled paper with seeds was put into sealed plastic bags to avoid moisture loss. All of the Petry dish irrigated by saline water (1dS/[m.sup.-1]).

Seeds were allowed to germinate at 25 [+ or -] 1[degrees]C for 7 days. Germination percentage was recorded after the 7th day. Germination percentage was calculated with the following formula:

Germination percantage (%) = Number of germinated seeds / Number of total seeds x 100

Also, Seedling vigor index was calculated by the following formula:

seedling vigor index = germination percentage x seedling dry weight

Data analyses were performed using the Spss statistical software (Version 15). Mean separations were performed by Duncan's multiple range test (DMRT) at 5% level.

## Results and discasion

The results showed that the effect of hydropriming was significant on germination in P # 0.05.

The seedling vigour, germination percentage and seedling dry weight increased by increasing in hydropriming time (Table 1).

Also, highest seedling vigour, germination percentage and seedling dry weight were achieved by hydropriming came up to 12 hours (Fig 1, 2 and 3).

Hydropriming clearly improved both rate of germination and mean germination time both under salt stress conditions. Furthermore, hydro priming resulted in increase of normal germination. The results are in line with the findings of [30] in Brassica and [38] in mustard. This process is important because allows the subsequent development of the embryo, especially in seeds characterised by a morphological dormancy (immature embryo), like *Chamaecyparis nootkatensis* seeds [56]. In many coated seeds, germination and subsequent seedling growth can be inhibited by mechanical restriction exerted by the seed coat [61]. In addition, high absorption of Na and Cl ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination and thus decreases germination percentage [63]. The exclusion of harmful ions (Na<sup>+</sup> and [Cl.sup.-]) from the shoot had already been found to be associated with genotypic variation in salt tolerance [47]. For those genotypes that cannot exclude toxic ions from the shoot, salt built-up to toxic levels in the leaves becoming the major cause of reduced growth [49]. Iqbal and Ashraf [29] reported that pretreatment with kinetin reduced Na<sup>+</sup> and increase K<sup>+</sup> in the shoot of salt tolerant cultivar under saline condition.

Aldequy and Ibrahim [3] observed that seed pre-treatment with GA<sub>3</sub>, IAA and ABA seemed to reduce stress by ameliorating Na<sup>+</sup>, [Cl.sup.-] and [P<sub>3</sub>.sup.+] and at the same time increasing K<sup>+</sup> levels within the developing grains. In the present study, increasing level of NaCl salinity increased Na<sup>+</sup> but decreased K<sup>+</sup> and Ca<sup>2+</sup> concentration in all three cultivars. Pre-treatment with ABA, BA and CCC caused an improvement in K<sup>+</sup> and Ca<sup>2+</sup> concentration in shoot of wheat seedlings. The magnitude of increase in K<sup>+</sup> concentration due to ABA was higher than BA and CCC pre-treated plants. Increase in Ca<sup>2+</sup> concentration due to ABA and BA pretreatment was statistically at par with each other in cv. Ca<sup>2+</sup> is important in preserving the integrity of the cell membrane during salt stress [52] and is used as secondary messenger in many signal transduction pathway within the cell [37]. Shabala et al. [58] reported that high Ca<sup>2+</sup> caused almost complete recovery of membrane potential root cells, which may be able to prevent K<sup>+</sup> leakage from the cell. Previous studies emphasized the important role of K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> for salt tolerance. Maathuis and Amtmann [39] emphasized that one of the key element in salinity tolerance is capacity to maintain a high cytosolic K<sup>+</sup>/Na<sup>+</sup> ratio because cytoplasmic Na<sup>+</sup> competes with K<sup>+</sup> binding sites and hence inhibits metabolic processes that crucially depend on K<sup>+</sup>. The maintenance of high K<sup>+</sup> and Ca<sup>2+</sup> content in salt tolerant cultivars may be one of the mechanisms underlying the degree of salt tolerance. Munns and James [47] suggested that variation in ion selectivity (e.g. K<sup>+</sup>) among genotypes of wheat is probably a secondary result of genetic variation in Na<sup>+</sup> uptake. Salt deposit in the root growing medium is the main reason for physiological drought and subsequently reduced cell division and/or enlargement in the root growing region and ultimately reduced root growth [49]. This is likely due to the interference of salinity stress on the phytohormones biosynthesis and action [14].

Furthermore, there is evidence that high levels of salinity inhibit growth of plants by retarding leaf primordia initiation [41]. Reduced leaf number and area lead to a low level of photosynthesis and photosynthate production and consequently lower plant production and biomass [2].

Ultimately, this trend led to an increased salinity tolerance of plants [2,27].

It has been reported previously that water deficit stress can result in reduced mobilisation of cotyledonary starch [34] and transport of sucrose from cotyledons to the embryonic axis [26].

Reduced growth and accumulation of sucrose in seedlings growing under stress conditions has been reported to be due to diminished activities of enzymes of sucrose breakdown [23]. Balibrea et al. [9] suggested that accumulation of sucrose due to lack of sucrolytic activity is responsible for the reduction of growth of tomato fruit under stress conditions. Increases in soluble sugars and sucrose in stressed seedlings might help in effective osmoregulation [25,59]. Exogenous application of GA3 and kinetin increased the growth of seedlings growing under water deficit stress conditions [32] by increasing the activities of enzymes of sucrose metabolism in shoots [34].

Kinetin significantly increases the rate of germination on most salinity levels [36]. Even at highest salinities, application of kinetin has proved to be effective in improving plant growth [71].

The possible mechanism of kinetin treatment has been reported, it was explained that the association between the internal mineral elements concentrations was largely affected by kinetin treatment. It reduced Na<sup>+</sup>, Ca<sup>2+</sup>, and Cl<sup>-</sup> accumulation and improved K<sup>+</sup> uptake under salinity stress. Increased K<sup>+</sup>/Na<sup>+</sup> ratio helped the plants to avoid Na<sup>+</sup> toxicity and enhanced shoot growth.

Kinetin also reduced membrane injury by dehydration and heat stressed and improved the water status of plants [7] this may be the reason for which the seedlings grown in our studies give improved growth under kinetin treatments. Ca<sup>2+</sup> is known to have an antagonistic effect on the uptake of Na<sup>+</sup> in plants subjected to NaCl stress and, thus, mitigate the toxic effect of Na<sup>+</sup> on plant metabolism [24,8]. There are a number of reports that show that increasing the Ca<sup>2+</sup> and K<sup>+</sup> concentration in seeds of different crops increases their germination in NaCl solutions, including wheat [13].

Ca<sup>2+</sup> could have a protective effect in root tips, which is of fundamental importance for the maintenance of root elongation in Na[Cl.sup.-]stressed seedlings [19]. An overall increasing trend in the catalase activity (units/ mg fresh weight) was observed except kinetin treatment in normal and chilling in saline conditions. Kinetin tends to increase the catalase activity above normal under salinity, as kinetin try to normalize the adverse effects of salinity [70]. Previously it has been reported that salinity stress increases catalase in wheat [55]. The adverse effects of salt stress on wheat seedling growth can be mitigated by presowing seed treatments particularly by chilling, kinetin and calcium chloride treatments.

Alleviation of adverse effects of salt stress by seed soaking treatments was associated with enhanced antioxidant capacity of the seedling.

[FIGURE 1 OMITTED]

[FIGURE 2 OMITTED]

[FIGURE 3 OMITTED]

Ascorbic Acid was detected in leaf and although its absolute level did not change in response to salt stress, the ascorbate/dehydro ascorbate ratios decreased progressively with the severity of stress.

However this antioxidative response does not seem to be sufficient to remove the harmful effects of high salinity [28] and similar trend has been observed in this study, because the growth of the seedlings was much affected by salinity than under normal conditions, when two controls were compared.

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Table 1: Means Comparison.

Hydro priming (Horse)	Germination percentage	Seedling dry weight(gr)	Seedling vigour
0	0c	0c	0c
6	79b	0.07b	5.53b
12	92.66a	0.08a	7.41a

Means within the same column and factors, followed by the same letter are not significantly difference.

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