Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 1, No. 4, p. 1-15, 2011
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Seed germination in response to osmosic stress

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Received: 03 May 2011
Revised: 27 June 2011
Accepted: 28 June 2011

Key words: Osmotic stress, seed germination, mungbean, cultivar.

Abstract

An Attempt was carried out to evaluate seed germination performances of Baraka, Adlib and Nineveh lentil cultivars besides Local Vetch and Local Mungbean cultivar under (0, -0.5, -1 and -1.5 Mpa) osmotic potentials created by dissolving pure NaCl in distilled water. Gradual reductions in osmotic solutions resulted in gradual reduction in all detected parameters. Subsequently, -1.5Mpa revealed the highest reductions in terms of final germination percentage (467.1%), germination rate (1710%), radical length (12829.4%) and Plumule length (infinity). It also aggravated the adverse effects by increasing the duration required for attaining the peak germination percentage (110.8%), as compared to that of distilled water. Treatments were categorized according to their adverse influence on performance of seed germinations as following: -1.5 Mpa> -1 Mpa> -0.5 Mpa> 0 Mpa. Mungbeans local cultivar seeds revealed the best germination performance, as compared to other pulse crops and their cultivars. Since this cultivar gave the highest germination rate (60.5 seedling.d^{-1}), plumule length (33 mm). In addition to that it significantly reduced days required for peak germination (2.6) and days to emergence commencements (1.3). Therefore, cultivars can be grouped according to their positively performance as below: Mungbean> Adlib>Nineveh> Baraka> Common Vetch. Mungbeans seeds appeared to be the most potent under all tested osmotic potentials. This cultivar manifested the highest plumule lengths under distilled water (108 mm), -0.5 Mpa (21mm) and -1.5 Mpa (3mm). Moreover this cultivar exhibited, days required for first emergence at all osmotic potentials.

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Introduction

Salt tolerance mechanism mainly preponderance by means that capable to excludes Na^+ from being in contact with functional enzymes to ensure enzyme inactivation. This task could be fulfilled by vast of gene expression, generate many enzymes responsible for transporting, sequestering and or secreting sodium ions throughout tissues. Glenn *et al.* (1999). Inge *et al.* (2009) postulated that modification of specific root Na^+ transport processes might improve Na^+ exclusion from the shoot and result, at least for some plants, in an increase in salinity tolerance. For example, initial influx of Na^+ from the soil could be decreased in the outer cell layers of the root, or efflux of Na^+ from these cells to the apoplast or soil solution could be increased. In the stele cells surrounding the vasculature, the loading of Na^+ into the xylem vessels could be decreased or retrieval of Na^+ from the transpiration stream increased. Accordingly, at the cellular level, Na^+ transport processes need to be modified in opposite directions in the inner and outer parts of the root to minimize Na^+ accumulation in the shoot. Consequently, plasma membrane Na^+ transport processes in the root need to be altered in a cell type–specific manner. Omami (2005) stated that under high salt concentration, plants sequester more NaCl in the leaf tissue than normally occurs. Increases in NaCl within the leaf tissue then result in lower osmotic potentials and more negative water potentials.

Under saline conditions, the osmotic adjustment, which occurs through the accumulation of inorganic compounds (mainly Na^+ and Cl^-) in plant, is less energy and carbon demanding than adjustment by organic solutes (Greenway and Munns, 1983). Maintenance of adequate levels of K^+ is essential for plant survival in saline habitats. Potassium is the most prominent inorganic plant solute, and as such makes a major contribution to the low osmotic potential in the stele of the roots that is a prerequisite for turgor pressure driven solute transport in the xylem and the water balance of plants (Marschner, 1995).

Water stress is usually created from water conductance constraints namely high osmosity at the rizophere, root absorption barriers, vessel conduit capability and stomata behaviours. Omami (2005) reported that the reduction in root hydraulic conductance reduces the amount of water flow from the roots to the upper portion of the canopy, causing water stress in the leaf tissue. However, (Shalhevet and Hsiao, 1986) found that the growth rate under water stress was half as large as under salt stress in the leaf water potential range of interest. Nevertheless, the deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Marschner, 1995). Sohan *et al.* (1999) revealed that osmotic effects of salt on plants are as a result of a lowering of the soil water potential due to increasing solute concentration in the root zone. Therefore, at very low soil water potentials, this condition interferes with plants ability to extract water from the soil and maintain turgour. Reduction of water uptake with salinity could be related to reductions in morphological and/or physiological parameters like leaf area, stomata density, and stomata closure (stomata conductance and transpiration). Since response to saline water varies greatly with species or cultivar (Bayuelo-Jiménez *et al.*, 2003).

Above 100 mM NaCl, the delay in the onset of germination was accompanied by reductions in the final germination percentage which decreased as the NaCl concentration increased. At NaCl concentrations of 200 mM and above, no germination was observed within 72 hrs of the start of imbibitions (Scorer *et al.,* 1985). They observed that NaCl greatly reduced the germination response of the seeds to R. The decreased R sensitivity observed in NaCl stressed seeds compares the influence response curves obtained with seeds germinated in water, 50 and 100 mM NaCl.

Germination tests were conducted under five osmotic potential levels (-0.45, -0.77, -1.03, -1.44 MPa, and Control) of PEG 6000 and NaCl. Germination percentage (%) at 4 and 8th days and also seedling growth traits such as root and shoot length (mm), dry root and shoot weight (mg), root: shoot length (R: S) ratio, and relative water content of shoot (RWC, %) were investigated in this study (Kaydan andYagmur, 2008).Their results indicated that decreases in the osmotic potentials caused a reduction in germination percentage and seedling growth. Seed germination completed in all seed size under control solution and at -0.45 MPa of NaCl at the 8^{th} day. Although, medium and small seeds had low germination percentage at the -0.77 MPa of NaCl, all large seeds germinated at the equivalent osmotic potential. However, subsequent low osmotic potentials of NaCl decreased germination percentage. Therefore, low germination percentage recorded at the highest NaCl concentration in all seed size. The objective of this investigation was to determine the germination performance of mungbeans, common vetch and three lentil cultivars under varying salt rates.

Materials and methods

This investigation was fulfilled at the laboratory of Field Crops Department, College of Agriculture, Salahalddin University, Erbil, Kurdistan Region, Iraq.

Factorial Randomized Complete Block Design was used in this experiment where factor (A) contained four osmotic potentials (0 Mpa (a_1), -0.5 Mpa (a_2), -1 Mpa (a_3, and -1.5 Mpa (a_4). Whereas factor (B) was represented by Nineveh lentil cv. (b_1), Adlib lentil cv. (b_2), Baraka lentil cv. (b_3), Local Common Vetch cv. (b_4) and Local Mungbean cv. (b_5). Subsequently, 20 treatments were included in this investigation. Every treatment was replicated 4 times and one replicate contained 4 plastic disposable dishes each of 25 seeds.

NaCl solutions was detected by electrical conductivity device and osmotic potential of the prepared solutions were calculated from Ayers and Wescot (1985) equation (Osmotic potential = - 0.36× ECe). 25 seeds were laid uniformly over salt wetted Watmann filter paper and sealed by polyethylene sheets to avoid seed desiccations. Germinated seeds were daily counted. Duration required for peak germination (days), and days for emergence commencements were counted. Final germination percentage, germination energy percentage were calculated from dividing number of germinated seeds on total seeds and from yield of number of germinated seeds after three days from starting divided on the total seeds, respectively, (Ruan *et al.,* 2002). Germination rate: germination percentage ratio was calculated from dividing the Germination rate over germination percentage. Radical and plumule lengths (mm) were measured by mini roller.

Germination rate (seedling.d^{-1}) was calculated from the following formula (Carleton, 1968): SG = No. of grains emerged at first count / Days of first count + ...+ No. of grains emerged at final count / days of final count. Mean germination time (days) was calculated from the equation below: ($MGR = \frac{\Sigma nidi}{N}$); where ni= number of germinated seeds on day I, d= rank order of day i (number of days counted from the beginning of germinated), and N=total number of germinated seeds. Finally, data were analyzed by computer statistical program, using Duncan's Multiple Range Test at α = 0.05 probability level. Finally permanent slides were prepared with some modification to that reported by Berlyn and Mksche (1976).

Results

Influence of NaCl concentrations

Germination of seeds under -1.5 Mpa (Table 1 and Fig. 1,a,b,c) profoundly inferior in all detected parameters, as compared to seeds germinating under distilled water (0Mpa) in terms of final germination percentage (467.1%), mean germination time (143%), germination energy (9300%), germination rate (1710%), ratio of germination rate to germination percentage (58.22%), radical length (12829.4%) and Plumule length (infinity). It also aggravated the adverse effects by increasing the duration required for attaining the peak germination percentage (110.8%), days required for first emergence (211.1%). When this treatment was compared with that of -0.5Mpa it also revealed substantially lower values in final germination percentage (438.5%), mean germination time (74.5%), germination energy (7925%), germination rate (1359.9%), ratio of germination rate: germination percentage (32%), radical length (1870.59%) and Plumule length (infinity). Additionally, this treatment revealed profound efficacies in increasing the period required for peak germination (60.8%) and days for first emergence (211%).

Table 1. Seed germination and seedling performances of Nineveh, Adlib, and Baraka lentil cultivars, Common Vetch and Mungbean in response to four osmotic potentials using NaCl Concentrations.

Treatments		Final Germination %	Mean Germination Time (days)	Germination Energy (%)	Germination Rate (seedling/day)	Days for Peak Germination
Osmotic Potential	0 Mpa	99.25a	1.665a	94.00a	56.40a	3.700d
	-0.5 Mpa	94.25b	1.195b	80.25b	45.478b	4.850c
	-1.0 Mpa	78.25c	1.283b	27.15c	23.473c	6.750b
	-1.5 Mpa	17.5d	0.685c	1.000d	3.115d	7.800a
Legume Crops	N	72.188a	1.356a	49.5b	26.963b	6.938a
	A	74.375a	1.316a	51.313b	27.325b	6.25b
	B	72.188a	1.278a	47.5c	24.988c	6.188b
	Common Vetch	69.688b	1.078b	35.0d	20.844d	6.938a
	Mungbean	73.125a	1.047b	69.688a	60.463a	2.563c
0 Mpa	N	97.5a	1.938a	93.75bc	47.425c	4.75d
	A	100.0a	1.438b	83.75de	38.6de	4.0de
	B	98.75a	1.025def	100.0a	100.0a	2.0f
	Common Vetch	100a	1.563b	87.5d	38.1de	7.5b
	Mungbean	100a	1.325bcd	92.5c	36.725e	4.0de
-0.5 Mpa	N	97.5a	1.063cf	80.0e	31.175f	4.75d
	A	92.5b	0.987f	41.25g	27.225g	6.0c
	B	88.75bc	1.088cf	100.0a	94.165b	2.0f
	Common Vetch	92.5b	1.35bc	16.75b	21.025h	7.5b
	Mungbean	100 a	1.363bc	15.25h	21.275h	8.0b
-1.0 Mpa	N	83.75d	1.263be	15.0h	19.575h	7.75b
	A	86.25cd	0.975ef	15.0h	13.80i	8.5ab
	B	83.75 d	1.463b	73.75f	41.688d	2.0f

	Common Vetch	62.5f	0.575g	0.0j	1.30k	8.0b
	Mungbean	75e	0.625g	0.0j	2.375jk	9.5a
-1.5 Mpa	N	10i	0.65g	0.0j	2.15k	8.0b
	A	18.75h	0.963ef	0.0j	3.75jk	9.25a
	B	17.5h	0.613g	5.0i	6.0j	4.25de
	Common Vetch	23.75g	0.963ef	0.0j	3.75jk	9.25a
	Mungbean	17.5h	0.613g	5.0i	6.0j	4.25de

Treatments		Days for First Emergence	Germination Rate: Germination % Ratio	Radical Length (mm)	Plumule Length (mm)
Osmotic Potential	0 Mpa	1.80c	0.568a	109.9a	62.35a
	-0.5 Mpa	1.80c	0.474b	16.75b	10.0b
	-1.0 Mpa	2.6b	0.31d	2.35c	1.9c
	-1.5 Mpa	5.6a	0.359c	0.85b	0.00d
Legume Crops	N	3.5a	0.311c	34.656b	15.875bc
	A	3.375ab	0.315c	36.625a	17.219b
	B	3.25b	0.313c	34.531b	14.656c
	Common Vetch	3.313b	0.49b	26.563d	12.063d
	Mungbean	1.313c	0.718a	30.0c	33.0a
0 Mpa	N	2.0e	0.485e	117.5b	52.5c
	A	2.0e	0.49e	121.25a	57.5b
	B	2.0e	0.475e	113.75c	51.25c
	Common Vetch	2.0e	0.388fg	88.75d	42.5d
	Mungbean	1.0f	1.0a	108.5d	108a
-0.5 Mpa	N	2.0e	0.378fg	18.0g	10.0f
	A	2.0e	0.398f	21.25f	9.75f
	B	2.0e	0.355g	20.0fg	5.5g
	Common Vetch	2.0e	0.295h	13h	3.75gh
	Mungbean	1.0f	0.945c	11.5h	21.0e
-1.0 Mpa	N	3.0d	0.253i	2.625ij	1.0hi
	A	3.0d	0.245i	3.0i	1.625hi
	B	3.0d	0.26hi	2.875ij	1.875hi
	Common Vetch	3.0d	0.22i	3.25i	2.0hi
	Mungbean	1.0f	0.573d	1.0k	3.0ghi
-1.5 Mpa	N	7.0a	0.13j	0.5k	0.0i
	A	6.5b	0.127j	1.0ijk	0.0i
	B	6.0c	0.123j	1.5ijk	0.0i
	Common Vetch	6.25bc	1.085a	1.25ijk	0.0i
	Mungbean	2.25e	0.355g	0.0k	0.0i

Table 2. Regression analysis results for the responses of germination performance to varying osmotic potential levels.

Character	Regression equation	(R^2)
Final Germination Percentage (%)	$Y = 99.25 - 21 X + 45.5 X^2 - 45 X^3$	96.7
Mean Germination Time (days))	$Y = 1.665 -2.326 X + 3.600 X^2 - 1.657 X^3$	58.1
Germination Energy (%)	$Y= 94 + 56.05 - 211.3 X^2 + 88.400 X^3$	84.9
Germination Rate (seedling/day)	$Y= 59.396 – 36.372 X$	57.2
Days for Peak Germination	$Y = 3.645 + 2.840 X$	39.7
Days for First Emergence	$Y= 1.120 + 2.440 X$	54
Germ. Rate:Germination Percentage Ratio	$Y = 0.546 – 0.158 X$	10.6
RadicalLength (mm)	$Y = 106.655 – 205.89 X$	95.5
Plumule Length (mm)	$Y = 62.35 – 174.317 + 164.6 X^2 – 50.733 X^3$	81.5

This treatment was followed by -1Mpa in sequence order, since the latter treatment significantly reduced the final germination percentage (26.8%), mean germination duration (29.8%), germination energy (246.2%), germination rate (140.3%), germination rate : germination percentage ratio (83.2%), radical length (4576.6%), and plumule length (3181.6%). This treatment also took similar trends in increasing the duration required for peak germination (82.4%) and days for first emergence (44.4%), as compared to treatment of distilled water media. The compression between –1Mpa to that of -0.5Mpa in term of final germination percentage (20.4%), mean germination duration (7.4%), germination energy (195.6%), germination rate (93.7%), germination rate: germination percentage ratio (52.9%), radical length (612.8%), and plumule length (426.3%). It highly increased the time required for peak germination (39.2%), days required for first emergence (44.44%).

Performance of seed germinations in -0.5Mpa manifested substantial reduction in relation to germinations performed under 0 Mpa in the final germination percentage (39.3%), germination energy (17.1%), germination rates (24%), germination rate: germination percentage ratio (19.8%), radical length (556.1%), and plumule length 523.5%). Moreover, it extended the period required for peak germination (31.1%). Subsequently, the best seed germination performance was obtained from seeds germinated in distilled water. These results suggested that germination of legume seeds under solutions higher than -0.5Mpa are not recommended owing to the risk of poor germination and low radical growth.

Fig. 1. Nature of germination and seedling performances of Mungbean in response to four osmotic potentials using NaCl concentrations.

Very close results were found by (Abdel, 1989). He germinated onion seeds in NaCl solutions at rates of 0, -5, -10 and -15 bars. Time required to first emergence, time to peak germinations, peak germination percentage, final seed germination, percentage of survived seeds after salt washing from un-germinated seeds revealed gradual substantial reduction confined with the gradual reductions in solute osmosity. These results were attributed to Na^+ and Cl^- toxic effects besides water imbibitions constraints. Fenugreek seeds germination capacity in varying NaCl solutions were highly reduced particularly under -1.5 MPa in compassion to distilled water check. Reductions were in terms of peak germination percentage (92%), and final germination percentage (94%). Salts influence on seed germination were attributed to the toxic effects of Na^+ and Cl^- preponderances in cellular membrane and cytosol by which enzymes are denaturized. Iyengar and Reddy (1996) found that salt toxicity caused particularly by Na+ and Cl- ions; and soil salinity represents an increasing threat to agricultural production. High sodium (Na^+) concentrations in soils are toxic to higher plants. More than 40% of irrigated lands worldwide show increased salt levels (Horie and Schroeder, 2004).

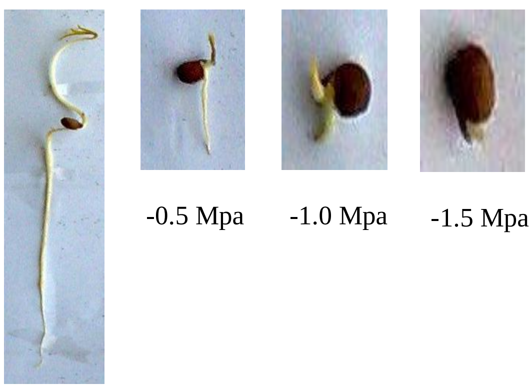

Fig. 2. Nature of germination and seedling performances of Common Vetch in response to four osmotic potentials using NaCl concentrations.

Cultivar responses

The obtained results (Table, 1 and Figure, 1, a,b,c) manifested that Mungbaen local cultivar seeds revealed the best germination performance, as compared to other pulse crops and their cultivars. Since this cultivar gave the highest germination energy (69.7%), germination rate (60.5seedling.d^{-1}), germination rate: germination percentage ratio (0.72), and plumule length (33 mm). In addition to that it significantly reduced days required for peak germination (2.6) and days to emergence commencements (1.3). Adlib lentil cultivar came next to local Mungbean in the superiority order. This cultivar was preponderated in germination energy (51.3%), germination rate (27.3seedling.d^{-1}), and plumule length (17.2 mm). Non- significant differences were observed between Adlib and Mungbean in final germination percentage, besides its overwhelming over all detected cultivars in radical length (36.5mm). The third cultivar in the sequence order was Nineveh cultivar which substantially exceeded Braka and Common Vetch in germination energy (4.2% and 41.4%, respectively) and germination rate (7.9% and 29.4%, respectively) and it highly exceeded Common Vetch in both radical length (30.5%) and plumule length (31.6%).

Fig. 3. Nature of germination and seedling performances of three lentil cultivars in response to four osmotic potentials using NaCl concentrations.

The fourth cultivar was Baraka as it showed superiority over Common Vetch in germination energy (35.7%) and (19.9%). Therefore, the worst cultivar was Common Vetch (Fig., 2 and 5). It revealed the lowest values in final germination percentage (69.7%), germination energy (35%), germination rate (20.8seedling.d^{-1}), radical length (26.6 mm) and plumule length (12.1mm). Cultivar differences in their capabilities of salt tolerance were well established. Unequivocal tolerance discrepancies among cultivars might be attributed to the individual cultivar genome expression ability, techniques that had been applied by producers and pollination contamination of mother plants in the field (Abdel, 2006).

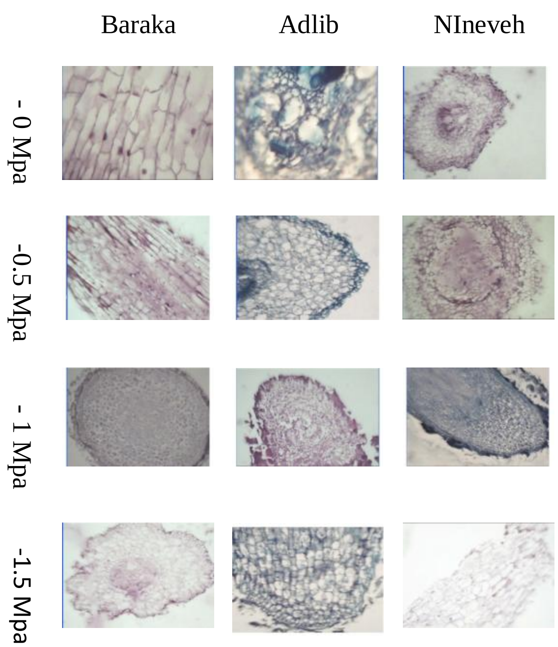

Fig. 4. The influence of varying osmotic levels on root anatomy of three lentil cultivars , Cell destructions are obvious, particularly at higher NaCl rates. (Magnification 7X40).

Varying responses between species were confirmed by Yousif et al. (2010). They examined the difference in the salt tolerance mechanisms between New Zealand spinach and water spinach (*Ipomoea aquatica* L.). Both plants were exposed to salt stress by daily irrigation with 0, 50, 100 and 200 mM NaCl solution for 14 days. They found that the growth of water spinach was markedly and gradually reduced with increasing salinity, whereas that of New Zealand spinach was increased with elevating salinity, indicating that New Zealand spinach is halophilic.

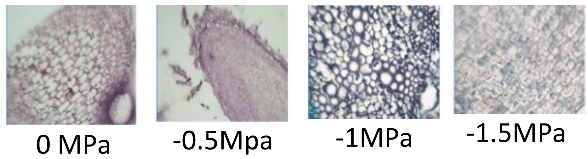

Fig. 5. The influence of varying osmotic levels on root anatomy of Common Vetch local cultivar. Cell destructions are obvious, particularly at higher NaCl rates. (Magnification 7X40).

Cultivar and osmotic solution interactions

Mungbean seeds appeared to be the most potent under all tested osmotic potentials (Table, 1& Figure, 1, 1a, b, c). This cultivar manifested the highest plumule lengths under distilled water (108 mm), -0.5 Mpa (21mm) and -1.5 Mpa (3mm). Moreover this cultivar exhibited the best germination rate: germination percentage ratio and days required for first emergence at all osmotic potentials (Fig. 3). The results also revealed that Adlib cultivar germination performance under distilled water, – 0.5 Mpa and -1Mpa was preponderance over all detected cultivars. It manifested the highest radical lengths (121.25 mm), (21.25mm) and (3 mm), respectively. It is worthy to mention Baraka cultivar overwhelming on all cultivar and all osmotic solutions in germination energy, germination rate, and lowest time for peak germination under 0, -0.5 and -1Mpa. Cultivar differences were obvious at the two highest potentials 0 and -0.5 Mpa. However, as the potential being decreased the variation among cultivar and /or species were gradually vanished. These results suggested that at high potential there were a chance to distinguish cultivars/and or species competitions. On the other hand when salt aggravated, plants lost their salt tolerance capabilities owing to overwhelming salt influences. Exiguously plant responses under low potential might be attributed to the effects of salts on cell metabolic (Fig. 1, 4), Amini and Ehsanpour (2005) germinated seeds of two tomato cultivars on medium containing only water agar, then transferred to MS medium supplemented with different concentrations of NaCl (0, 40, 80, 120 and 160 mM) for 21 days. They manifested that increasing of salinity resulted in increasing of soluble proteins in stem and leaf of cv. Isfahani but decreasing in cv. Shirazy. Soluble proteins in roots of both cultivars showed some variations.

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