

PRIMING-INDUCED METABOLIC CHANGES IN THREE ANNUAL MEDICS SPECIES IMPROVE GERMINATION AND EARLY GROWTH UNDER DROUGHT AND SALT STRESS CONDITIONS

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Summary. The effects of seed priming chemicals such as urea and potassium nitrate on proline content, germination and early growth characteristics of three annual medics plants under drought and salt stress were studied in controlled conditions during 2010-2011. Treatments consisted of stress type and intensity at five levels: moderate drought (MD), severe drought (SD), moderate salt (MS), severe salt (SS), and control (C, without stress). Seed priming was applied at three levels: water (control), KNO₃ and urea. Three annual medics species (*Medicago scutellata*, *M. rigidula* and *M. polymorpha* L.) were used in the study. The results showed that the highest germination percentage (Ger %), germination rate (GR), seedling length (SL), radical length (RL) and seedling to radical length ratio (S/R) were achieved in control treatments while the highest proline and protein contents were measured in the severe drought treatment. Urea priming led to higher Ger %, GR, SL and RL followed by KNO₃ priming. Priming had no effect on S/R and proline content. Among the three annual medics species studied *Medicago scutellata* showed the highest Ger %, GR, SL, RL, proline and protein contents. Salt stress affected Ger %, GR and RL to a higher extent than drought stress whereas drought stress affected S/R more strongly than salt stress. Both types of stress decreased all measured parameters except for protein and proline contents that were increased remarkably. Drought stress increased proline content more strongly than salt stress. Generally, in both drought and salt stress conditions, potassium nitrate and urea led to increased Ger %, GR, SL and RL. Therefore, seed priming can be recommended to combat the effects of drought and salt stress.

Key words: Germination; potassium nitrate; priming; proline.

Abbreviations: Ger % – Germination percentage; GR – germination rate; SL – Seedling length; RL – Radical length; S/R – Seedling to radical length ratio.

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INTRODUCTION

Periodic and unpredictable environmental stresses force plants to adapt and this has been the case since the time of early migration from aquatic environments to the land. Soil moisture deficiency and salinity affect crop production in all arid and semi-arid regions of the world (EL-Siddig *et al.*, 1998). The major limiting factor in most regions where plants are subjected to extreme water deficit during dry seasons is salinity in the irrigation water (Kerepesi and Galiba, 2000). Because of high evaporative demand and insufficient leaching of ions due to low precipitation in these climatic conditions, salts may be accumulated in the soil. Improved salt tolerance of crops can lower the leaching requirement, and so reduce the costs of an irrigation scheme, both in the need to import fresh water and to dispose saline water. Salt-tolerant crops have a much lower leaching requirement than salt-sensitive ones. In dry-land agriculture, improved salt tolerance can increase yield on saline soils (Gucci *et al.*, 1997).

Medicinal plants, (Derkaoui *et al.*, 1993; Zhu *et al.*, 1996) and high seed productivity (Conway *et al.*, 2001), are considered potential species for pasture improvement in semi-arid zones (Meloni *et al.*, 2000) due to their high-quality forage for livestock.

At any stage of crop growth, moisture stress can cause an irreversible loss in yield potential (Reginato, 1993). An essential prerequisite to reach the yield potential, quality, and ultimately profit in annual crops is the rapid and uniform field emergence (Parera and Cantliffe, 1994). For achieving an optimal seedling

establishment and better productivity, we need greater and better synchronized germination, however, several environmental constraints are great impediments (Wahid *et al.*, 2008).

To increase crop production one pragmatic approach is seed invigoration (Lee and Kim, 2000; Basra *et al.*, 2004; Farooq *et al.*, 2006). Strategies for seed invigoration include hydropriming, osmoconditioning, osmohardening, hardening, hormonal-priming, matri-priming, osmo-priming (soaking in osmotic solutions such as polyethylene glycol, potassium salts, e.g., KCl, K₂SO₄), solid matrix priming and using plant growth regulators (PGRs) (Dearman *et al.*, 1987; Capron *et al.*, 2000; Chiu *et al.*, 2002).

Priming offers to raise seed performance in many crop species (Chiu *et al.*, 2002). It allows seeds to imbibe to proceed to the first stage of germination. Seed priming is pre-sowing treatment in osmotic solution, however, the treatment prevents radical protrusion through the seed coat (Heydecker *et al.*, 1973).

A viable technology to enhance rapid and uniform emergence is priming (Dearman *et al.*, 1987; Parera and Cantliffe, 1994). A common seed treatment to reduce the time between seed sowing and seedling emergence was seed priming (Parera and Cantliffe, 1994). Loss of membrane integrity, changes in enzymatic activities, decline in protein and nucleic acid synthesis, and lesions in DNA caused seed deterioration (McDonald, 1999). The production of active oxygen species depends frequently on these deteriorative changes (Bernal-Lugo and Leopold, 1998; McDonald, 1999).

Researchers have reported that we

can improve germination percentage and increase seedling establishment and growth by seed priming (Basra *et al.*, 2003; Mohammadi, 2009). Little information is available in literature on the effect of seed priming on germination and early growth rate of annual medics plants.

The present study was conducted to examine the effects of different seed priming chemicals such as urea and potassium nitrate on total protein, proline content, germination percentage and seedling growth characteristics of three annual medics plants under drought and salt stress conditions.

MATERIALS AND METHODS

This study was carried out as a factorial experiment based on a completely randomized design in a controlled environment in the laboratory of the college of agriculture, Shiraz University, Shiraz Iran (52°46' E, 29°50' N, altitude 1810 mASL), 12 km north of Shiraz during 2010-2011. The first factor was stress type and intensity at five levels; moderate drought, severe drought, moderate salt, severe salt and control (without stress). Seed priming was the second factor; water as control, potassium nitrate and urea,

and species of annual medics plants were the third factor; *Medicago scutellata*, *M. rigidula* and *M. polymorpha* L. In this study, measurements of proline content (Pro), germination percentage (Ger %), germination rate (GR), seedling length (SL), radical length (RL) and seedling to radical length ratio (S/R) were done. Seedpods were handpicked and the seeds were separated from the pods by hand and stored in paper bags at room temperature (25°C) until the germination tests were performed.

Before planting, the seeds were primed with the specified primer for 24 h. The seeds were placed in 9 cm Petri dishes on two layers of filter paper (Whatman No.1). Twenty five seeds were placed in each Petri dish.

The Petri dishes were irrigated with five different solutions. The following treatments were applied: moderate drought (MD), severe drought (SD), moderate salt (MS), severe salt (SS) and without stress (control, C). The solutions used are shown in Table 1. NaCl was used for salt stress treatment and PEG6000 for drought stress treatment (Ellis *et al.*, 1987). The dishes were placed in a germinator at 20-25°C. The filter papers of each Petri dish were replaced every two days to prevent salt

Table 1. Types of stress used in the experiment.

Solution	Made state
Moderate Drought	-0.1 bar*
Severe Drought	-0.5 bar
Moderate Salt	50 mM NaCl
Severe Salt	100 mM NaCl
Control	Without stress

*Based on Michel and Kaufmann formula (Michel and Kaufmann, 1973) by using of PEG 6000.

accumulation (Rehman et al., 1998).

Seed germination was recorded daily up to day 11 after the beginning of the experiment. A seed was considered germinated when the radical emerged 2 mm in length. In each measurement, four seedlings were randomly selected from each Petri dish and their averages were considered as sample data.

Proline content was determined according to the acid-ninhydrin method (Bates et al., 1973) with some modifications. Frozen samples (approximately 0.2 g) were homogenized in 10 ml of 3% (v/v) aqueous sulfosalicylic acid. The homogenate was filtered through Whatman 41 filter paper. The filtrate, acid-ninhydrin agent and glacial acetic acid (1 ml each) were heated for 1 h at 100°C (Table 1). The reaction mixture was extracted with 5 ml toluene. The absorbance was measured at 520 nm in the upper toluene layer. Protein content was measured according to Lowary et al. (1951).

Statistical analysis was performed for each parameter based on a randomized complete block design model with four replications using MSTATC software (Edward, 1986). Means were separated by application of Duncan's Multiple Range Test at $p \leq 0.05$.

RESULTS

Germination

The germination percentage was significantly affected by stress, priming, species and interactions between stress and priming, stress and species, priming and species and also interaction of stress, species and priming (Table 2). The highest germination was achieved in the control (99.17%) and the least severe salt treatment (42%) (Table 3). Salt stress affected germination more drastically than drought stress. Among priming treatments, germination percentage was higher upon urea priming (Table 3).

Table 2. Mean squares for measured characteristics.

SOV	df	Ger. %	GR	SL	RL	SL/RL	Proline	Protein
S	4	11357.0555**	122.9730**	1654.0191**	8711.0940**	0.6894**	17.8465**	2968.1636**
P	2	2236.5722**	4.6165**	697.7202**	7846.1545**	0.3042**	7.2438**	7931.3522**
Sp	2	1346.2500**	2.8933**	4.2662 ^{ns}	618.1760**	0.1029 ^{ns}	5.7720**	190.0563*
S×P	8	435.4680**	1.6531**	97.5375**	314.4621**	0.2327**	1.5832**	736.6960**
S×Sp	8	187.3333**	1.7816**	6.0778 ^{ns}	235.0780**	0.0038 ^{ns}	1.6793**	45.0713*
P×Sp	4	146.6166**	1.3248**	3.3511 ^{ns}	376.0975**	0.0012 ^{ns}	0.5638**	789.0613**
S×P×Sp	16	247.5125**	1.2807**	3.8862 ^{ns}	368.3056**	0.0035 ^{ns}	0.2873**	15.4587 ^{ns}
Error	90	16.5722	0.8950	5.2489	39.6151	0.0038	0.1563	73.2156
CV (%)		6.4209	11.2847	7.5947	9.0431	14.9215	20.8478	17.0876

*, ** and ^{ns} – Significantly different at 5% and 1% probability level, and no significant difference, respectively.

S – Stress; P – Priming; Sp – Species; Ger. % – Germination percentage; GR – Germination rate; SL – Seedling length; RL – Radical length.

Among the species, germination percentage was higher in *M. scutellata*, as shown in Fig. 1. This result can be useful for farmers to achieve better seedling establishment in *M. scutellata* under undesirable conditions.

In control conditions, no difference in germination was observed between the primed and the non-primed treatments. However, in moderate and severe drought stress urea priming resulted in the best germination compared to the other primers.

Effects of stress, priming and interactive effects of stress and priming on the germination rate were highly significant (Table 2). The highest germination rate was achieved in the control. Salt stress caused a stronger decrease than drought and the germination rate was higher in moderate rather than severe stresses conditions. Urea priming resulted in a higher germination rate and there was no significant difference between potassium nitrate and water priming (control). Although there was significant difference

Table 3. Mean comparison for the main effects of treatments on proline content, germination percentage and seedling growth.

	Ger. %	GR	SL [mm]	RL [mm]	SL/RL	Proline [$\mu\text{mol/g DW}$]	Protein [mg/g DW]
Stress							
C	90.17 ^a	4.70 ^a	13.76 ^a	75.00 ^a	0.47 ^a	1.92 ^b	281.25 ^c
MD	78.33 ^b	2.72 ^b	9.04 ^b	78.55 ^a	0.11 ^c	1.76 ^b	371.25 ^b
SD	62.00 ^c	1.22 ^c	2.88 ^c	53.45 ^c	0.07 ^d	2.57 ^a	494.23 ^a
MS	72.58 ^b	1.48 ^d	10.23 ^b	57.80 ^b	0.36 ^b	1.35 ^c	293.82 ^c
SS	41.80 ^d	0.67 ^e	2.27 ^c	43.20 ^d	0.09 ^d	1.27 ^d	271.42 ^d
Priming							
Water	75.50 ^b	2.52 ^b	6.04 ^c	31.72 ^c	0.46 ^a	3.17 ^a	306.85 ^b
KNO ₃	78.00 ^b	2.27 ^b	15.43 ^b	65.60 ^b	0.25 ^b	1.58 ^b	296.09 ^c
Urea	83.00 ^a	4.69 ^a	24.76 ^a	71.56 ^a	0.28 ^b	1.17 ^b	382.14 ^a
Species							
<i>M. scutellata</i>	85.93 ^a	7.43 ^a	15.58 ^a	82.00 ^a	0.31 ^a	1.79 ^a	344.13 ^a
<i>M. rigidula</i>	58.27 ^c	2.55 ^c	12.97 ^b	66.28 ^c	0.30 ^a	1.31 ^b	268.30 ^c
<i>M. polymorpha</i>	63.40 ^b	3.89 ^b	12.81 ^b	72.68 ^b	0.23 ^a	1.18 ^b	290.49 ^b

Means with the same letter in each column are not significantly different using Duncan's Multiple Range tests ($p \leq 0.05$).

(Control – Without stress; MD – Moderate drought; SD – Severe drought; MS – Moderate salt; SS – Severe salt)

Ger. % – Germination percentage; GR – Germination rate; SL – Seedling length; RL – Radical length.

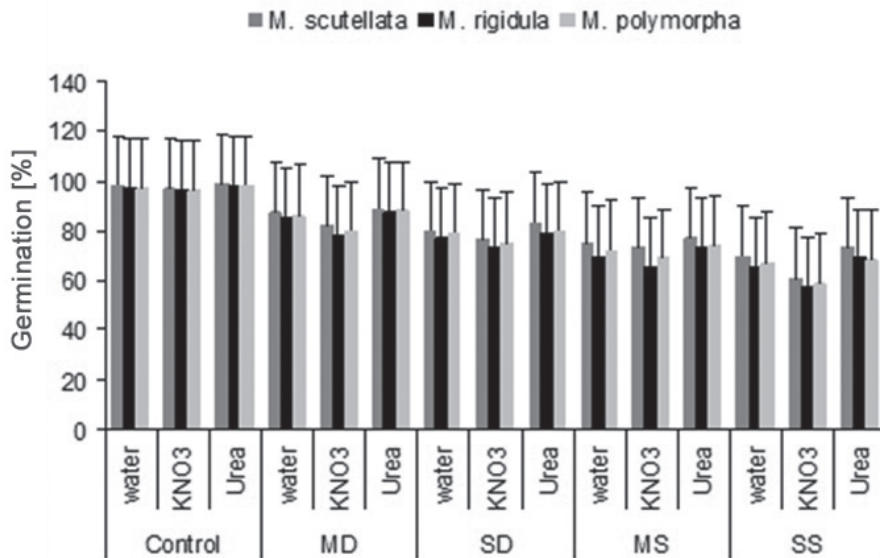


Fig. 1. Relation between stress (Control – without stress; MD – moderate drought; SD – severe drought; MS – moderate salt; SS – severe salt) and priming (urea and KNO₃) on germination percentage of three medic species (*Medicago scutellata*, *Medicago rigidula* and *Medicago polymorpha* L.). Least Significant Difference = 6.3421.

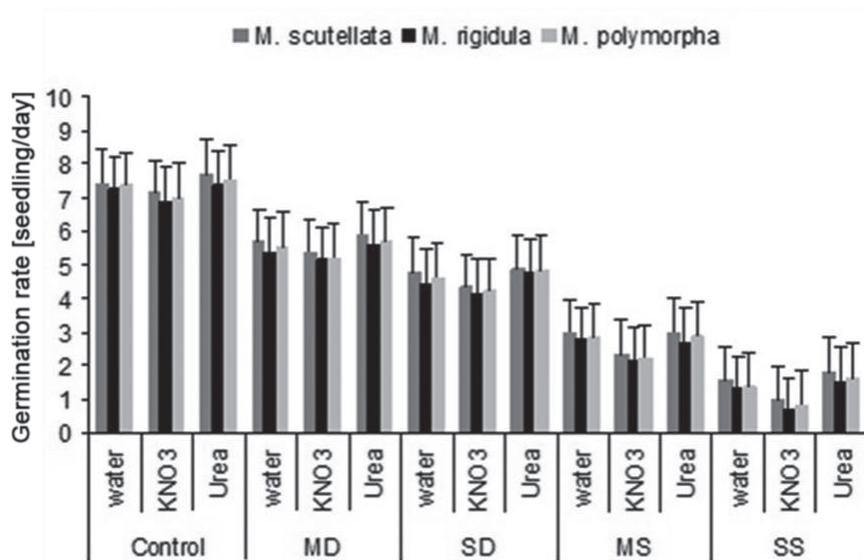


Fig. 2. Relation between stress (Control – without stress; MD – moderate drought; SD – severe drought; MS – moderate salt; SS – severe salt) and priming (urea and KNO₃) on germination rate of three medic species (*Medicago scutellata*, *Medicago rigidula* and *Medicago polymorpha* L.). Least Significant Difference = 0.9869.

between the species, *M. scutellata* showed a better germination rate (Fig. 2). This trait facilitates the faster emergence of annual medics forming a complete canopy at early growth. Considering the interaction of stress and priming, it was notable that urea affected most positively the germination rate.

Biochemical changes

Protein level significantly affected stress, priming, interaction of stress and priming, interaction of priming and species (99%), species and interaction of stress and species at 95% probability level (Table 2). The highest and the least protein levels were observed in severe drought and severe salt stress, respectively. The protein level was higher under the urea priming treatments as shown in Fig. 3.

This may be due to the role of nitrogen in protein structure. Protein content in *M. scutellata* was higher compared with the other hybrids.

Priming, stress, species and their interaction had a significant effect on proline content. The highest proline content was measured after severe drought compared to moderate drought. Salinity affected negatively proline content. At slightly more negative water potentials, the amino acid proline begins to increase sharply. In the present study, both priming agents decreased proline content. Control treatments resulted in a higher proline level compared with urea and potassium nitrate. Among the species tested, *M. scutellata* showed the highest proline content, indicating that this species might be more resistant to drought (Fig. 4).

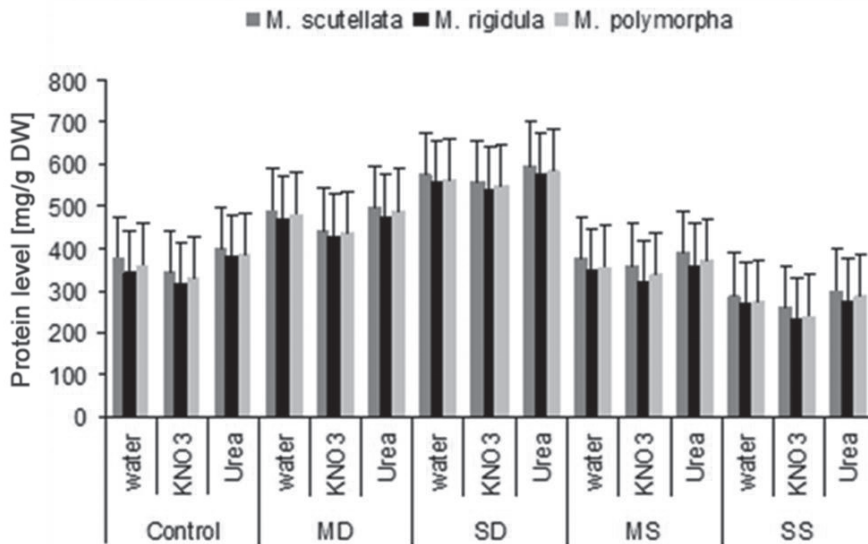


Fig. 3. Relation between stress (Control – without stress; MD – moderate drought; SD – severe drought; MS – moderate salt; SS – severe salt) and priming (urea and KNO₃) on protein content [mg/g DW] of three medics species (*Medicago scutellata*, *Medicago rigidula* and *Medicago polymorpha* L.) - Least Significant Difference = 0.798.

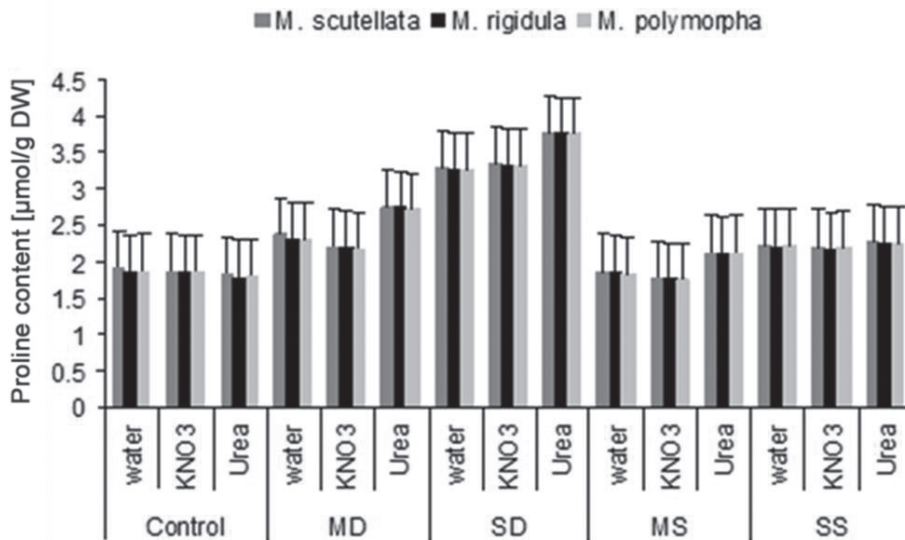


Fig. 4. Relation between stress (Control – without stress; MD – moderate drought; SD – severe drought; MS – moderate salt; SS – severe salt) (drought and salt) and priming (urea and KNO₃) on proline content [µmol/g DW] of three medic species (*Medicago scutellata*, *Medicago rigidula* and *Medicago polymorpha* L.). Least Significant Difference = 0.599.

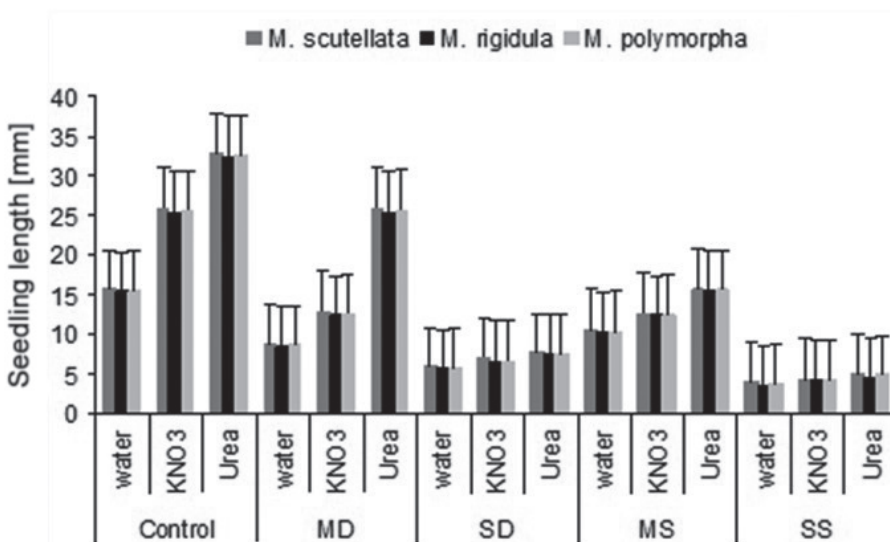


Fig. 5. Relation between stress (Control – without stress; MD – moderate drought; SD – severe drought; MS – moderate salt; SS – severe salt) and priming (urea and KNO₃) on seedling length [mm] of three medic species (*Medicago scutellata*, *Medicago rigidula* and *Medicago polymorpha* L.). Least Significant Difference = 2.8769.

Early growth

Seedling length was affected by stress and priming as well as the interactions of stress and priming (Table 2). The longest seedlings were recorded in the control treatment whereas the shortest seedlings were measured under severe drought and salt stress. Priming had a positive effect on seedling length and urea was more effective than potassium nitrate (Table 3). Seedling length decreased due to salt stress, but the level was higher than under drought stress (Fig. 5).

All sources of variance had a significant effect on radical length. Although the stress treatments decreased the radical length, the decrease in the radical length was less than that of the seedling length. Priming agents had a positive effect on radical length and urea was found better than potassium nitrate, as

shown in Fig. 6. *M. rigidula* showed the lowest radical length. Priming decreased the seedling to radical length ratio and it was lower than 1 in all treatments.

DISCUSSION

In this study, salt stress decreased more strongly the germination percentage, germination rate, seedling length, radical length and the seedling to radical length ratio. At any stage of crop growth such as germination, moisture stress can cause an irreversible loss in yield potential (Reginato, 1993). Both inhibition or delayed seed germination and seedling establishment resulted from salt and osmotic stresses (Almansouri et al., 2001). There is a decrease in water uptake during imbibition and furthermore salt stress may cause an

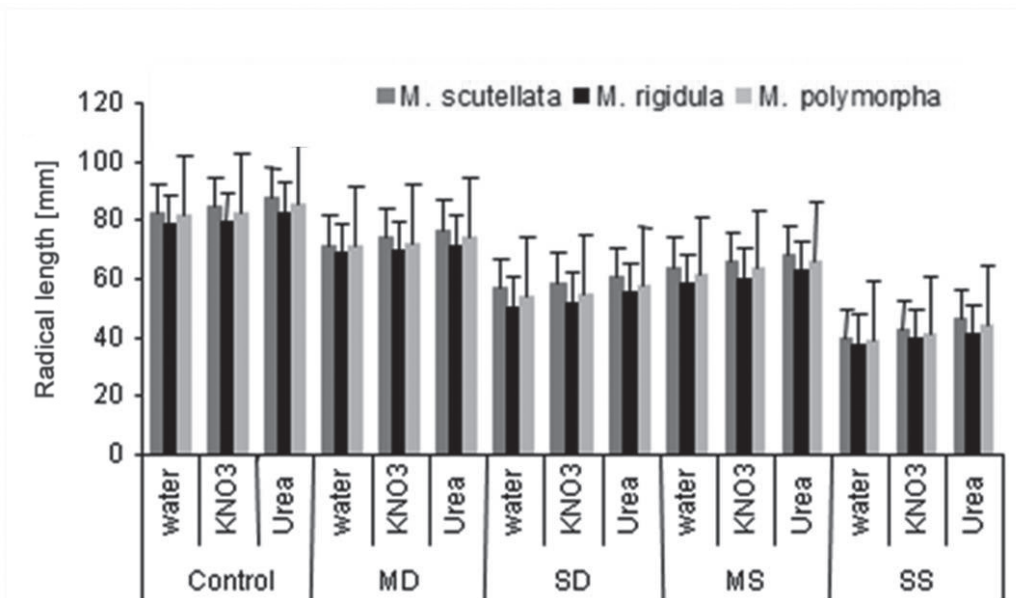


Fig. 6. Relation between stress (Control – without stress; MD – moderate drought; SD – severe drought; MS – moderate salt; SS – severe salt) and priming (urea and KNO₃) on radical length [mm] of three medics species (*Medicago scutellata*, *Medicago rigidula* and *Medicago polymorpha* L.). Least Significant Difference = 8.9987.

excessive uptake of ions under these stresses (Murillo-Amador et al., 2002). Critical stages in a plant's life cycle are germination and seedling establishment (Emam, 2007). Plant density, uniformity and management options were determined as crop production and stand establishment (Cheng and Bradford, 1999). At the early growth stage, a good rate of germination helps annual medic to fast emergence and canopy closure. Seed priming has been used to improve germination, reduce seedling emergence time and improve stand establishment and yield (Murungu et al., 2004).

Research has demonstrated that in many crops improved germination has been achieved after seed priming (Parera and Cantliffe, 1994; Singh, 1995). Under stressful conditions seed priming has enhanced seedling establishment (Ashraf and Rauf, 2001; Basra et al., 2004). Determinate priming rate is very important because seedling growth of rapeseed reduces at a high level seed priming (Omidi et al., 2005) and high grain yield is not necessarily obtained from a high level of prime (Subedi et al., 2005). However, longevity of primed seeds was decreased (Chiu et al., 2002).

Our results showed that both stresses increased proline and protein contents and the effect of salt stress was stronger than drought stress. Urea priming increased the germination percentage, germination rate, seedling length, radical length, the seedling to radical length ratio and protein content remarkably. Potassium nitrate ranked second. Thus, for normal and stress conditions, it is recommended that farmers use urea and potassium nitrate priming, respectively. Between species, *M. scutellata* showed

the best traits under control conditions as well as the best response under stress conditions. Water stress may be less effective on protein synthesis (Reginato, 1993). Potassium nitrate priming had a more positive effect than water priming. Protein content in *M. scutellata* was higher compared with the other species.

Proline content decreased under salt stress, the decrease being stronger in severe salt stress treatments. Protein synthesis may increase under water stress. The amino acid proline begins to accumulate sharply due to less availability of water. Free proline accumulation has been suggested to be an indicator of drought resistance (Emam and Seghatoeslami, 2005). Urea priming at all stress levels increased proline content because of the role of nitrogen in the structure of proline (Reginato, 1993).

Overall, the germination percentage was significantly affected by stress, priming, species and interactions between stress and priming, stress and species, priming and species and also the interaction between stress, species and priming. In control conditions, no difference in germination between the primed and the non-primed treatments was found. However, in moderate and severe drought stress urea priming led to the best germination compared to the other primers. The highest levels of proline and protein were found under drought stress conditions. Generally, in conditions of both drought and salt stress potassium nitrate and urea alleviated the negative effects and led to increased germination as well as seedling and root lengths. Thus, seed priming can be recommended for stress conditions.

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