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Halopriming and Hydropriming Treatments to Overcome Salt and Drought Stress at Germination Stage of Corn (*Zea mays* L.)

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Abstract

To study the effects of halopriming and hydropriming in overcoming salt and drought stress in corn (*Zea mays* L.), two experiments were separately conducted at Shahrood University of Technology. Seed treatments consisted of control (untreated seeds), soaking in distilled water for 32 h (hydropriming), and soaking in 50 mmol solution of $CaCl_2$ for 16 h (halopriming). Germination and early seedling growth were studied using distilled water (control) and osmotic potentials of -0.4, -0.8, and -1.2 MPa from NaCl (salinity stress) and polyethylene glycol [PEG 6000 (drought stress)]. Results showed that in both salinity and drought experiments, germination percentage reduced significantly according to decreased osmotic potential. Hydroprimed and haloprimed seeds achieved the minimum reductions in germination percentage. The maximum reduction in germination percentage was recorded from untreated seeds (control). Minimum reduction percentages of root length (RPL root) and shoot length (RPL shoot) were attained from hydroprimed and haloprimed seeds due to NaCl and PEG conditions (-0.4 MPa), and maximum RPL root and RPL shoot were attained from controlled seeds due to NaCl and PEG (-1.2 MPa) conditions. The reduction percentage of dry weight for root (RPD root) and shoot (RPD shoot) increased according to increased osmotic potential in both NaCl and PEG, but RPD for shoot was significantly affected compared with RPD for root. Interaction of seed priming treatment and osmotic potential for the germination index (GI) showed that under 0 and -0.4 MPa, hydroprimed and haloprimed seeds had higher GI as compared with untreated seeds due to NaCl and PEG conditions. Interaction between the seed priming treatment and osmotic potential significantly affected the vigour index (VI) due to NaCl and PEG conditions, and halopriming significantly increased VI at high osmotic potentials. On average, the VI of haloprimed seeds was higher than that of untreated seeds at high osmotic potentials and was not significantly different from hydroprimed and untreated seeds at low osmotic potentials. It is concluded that under salinity stress, the osmotic effect is more important than the toxic effect in loss of seed germination. Moreover, hydropriming practically ensured rapid and uniform germination with few abnormal seedlings.

Keywords: Seed establishment; Germination indices; Seedling growth

1. Introduction

Drought and salinity are widespread stresses around the world. Plant responses to salt and drought stress have much in common. According to various studies, 7% of the world's land is saline, 3% of which has high salinity because of low precipitation, high evaporation, and irrigation with saline water. Soil salinity is increasing (Teimouri *et al*., 2009). Poor germination and decreased seedling growth result in poor establishment and, occasionally, crop failure. Salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate along with a suite of metabolic changes identical to those caused by water stress. Salinity and drought are common abiotic stress factors seriously affecting crop production in different regions, particularly in arid and semi-arid regions. Salinity can affect germination and seedling growth either by creating an osmotic pressure (OP) that prevents water uptake or through the toxic effects of sodium and chloride ions on the germinating seed (Bewley and Black, 1982). The most important problem causing low economic crop

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yield in arid regions is the high concentration of ions, especially NaCl, in either soil or irrigation water (Moeinrad, 2008). The low economic yield can be attributed to the crop's susceptibility to a number of biotic and abiotic stresses, among which, and of alarming concern, are salt and drought stress. High salinity adversely affects germination, growth, physiology, and productivity by causing ionic and osmotic stresses as well as oxidative damage (Iterbe-Ormaetxe *et al*., 1998).

One way to exploit the large areas of saline soils and saline water sources is to improve salt tolerance in cultivated plant species. The adverse effects of high salt concentrations for plants are caused by the osmotic retention of water and by specific ionic effects on the protoplasm. Water is osmotically held in salt solutions; so, as the concentration of salt increases, water becomes less and less accessible to the plant. An alternative strategy for possibly overcoming salt and drought stresses is treating seeds with hydropriming or other treatments. Seed priming is defined as pre-sowing treatments in water or in an osmotic solution that allows the seed to imbibe water in order to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat. Typical responses to priming are faster and closer spreads of time to germination, emergence over all seedbed environments, and wider temperature ranges of germination, leading to better crop stands, and hence improved yield and harvest quality, especially under suboptimal and stress growing conditions in the field (Parera and Cantliffe, 1994; Singh and Rao, 1993; Sadeghian and Yavari, 2004). Seed priming is beneficial in many respects. Seed priming techniques such as hydropriming, hardening, osmoconditioning, osmohardening and hormonal priming have been used to accelerate the emergence of roots and shoots, produce more vigorous plants, and develop better drought tolerance in many field crops (Abbasdokht and Edalatpishe, 2013). The most important priming treatments are hydropriming and halopriming. Seed priming has been successfully demonstrated to improve germination and emergence in the seeds of many crops, particularly vegetables and small seeded grasses (Bradford, 1986). Dharmalingam and Basu (1990) reported the beneficial effects of a hydration-dehydration seed treatment on germination of sunflower.

Previous works (Afzal *et al*., 2005; Ashraf and Rauf, 2001; Basra *et al*., 2006; Roy and Srivastava, 2000) have suggested that the adverse and depressive effects of salinity and water stress on germination can be alleviated by various seed priming treatments. Although the effects of priming treatments on germination of some seed crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur *et al*., 2002), sunflower (Kaya *et al*., 2006), and cotton (Casenave and Toselli, 2007) have been studied, relatively little information is available on the corn seed under salt and drought stress in Iran. The effects of halopriming and hydropriming to overcome salt and drought stress in corn (*Zea mays* L.) should be investigated not only during germination, but also during sensitive growth stages.

2. Materials and Methods

Two experiments were separately carried out at the Seed Research Laboratory, Shahrood University of Technology, Shahrood, Iran. Factorial combinations of S.C. 704 cultivar of corn (primed and non-primed) and 3 salinity levels (-0.4, -0.8, and -1.2 MPa) were created using NaCl according to the Vant Hoff formula presented in Salisbury and Ross (1996), and drought stress levels of -0.4, -0.8, and -1.2 MPa as treatments were created using polyethylene glycol (PEG 6000) based on the equation supplied by Michel and Kaufman (1983). The experimental design was a randomized complete design with four replications for each experiment.

Seed priming treatments consisted of T1: control (untreated seeds), T2: soaking in distilled water for 32 h (hydropriming), and T3: soaking in 50 mmol solution of $CaCl_2$ for 16 h (halopriming). Priming treatments were conducted separately at 20°C in the dark and redried to original weight with forced air under shade following the procedure of Basra *et al*., (2006). NaCl concentrations had electrical conductivity (EC) values of 3.7, 12.3, 17.4, and 21.8 dSm^{-1}. The referred osmotic potentials of NaCl solutions (-0.4, -0.8, and -1.2 MPa) were prepared using 5.25, 10.5, and 15.75 grams of NaCl per liter of distilled water. The osmotic solution of PEG was prepared using 161, 241, and 302 gram of PEG per liter for -0.4, -0.8, and -1.2 MPa, respectively. Four replications of 25 primed and non-primed seeds were germinated in 12 cm in diameter glass Petri dishes at 25±1°C in a dark growth chamber with 45% relative humidity. 10 ml of each osmotic solution was added to each Petri dish, and seed germination was scored when root length reached 2 mm or more. Germinating seeds were counted daily and terminated when no further germination occurred. Germination percentage (GP) was calculated using the formula below for the treatments in each replication (Scott *et al*., 1984):

GP=[Total seeds germinated (when no further germination occurred)/Total number of seeds] ×100

The reduction percentage of germination (RPG) and reduction percentage of emergence (RPE) were calculated according to the following formula (Madidi *et al*., 2004):

$RPG\ (or\ RPE) = \{1 - (NX / NC)\} \times 100$

NX is the number of germinated (or emerged) seedlings under the salt treatment, and NC is the number of germinated (or emerged) seedlings in the control. For each replicate, the reduction percentage of root or shoot length (RPL) was calculated according to the following formula (Madidi *et al*., 2004):

$RPL = \{1-(\ell x/\ell c)\} \times 100$

where ℓx and ℓc are the mean values of root or shoot length recorded in the stressed and normal treatments, respectively.

The reduction percentage of dry weights (root or shoot) was calculated according to the following formula (Madidi *et al*., 2004):

$RPD = \{1-(Y/X)\} \times 100$

where Y and X are the mean values of dry weights recorded in the stressed and normal treatments, respectively.

Vigour index (VI) was calculated using the formula of Abdul-baki and Anderson, (1973):

$VI = [MSL + MRL] \times GP$

where MSL is mean shoot length, MRL is mean root length, and GP is germination percentage (%).

The germination index (GI) which is expressed as speed of germination was calculated as described in Scott *et al*., (1984):

$GI=\sum (TiNi)/S$

where Ti is the number of days after sowing, Ni is the number of seeds germinated on the *i*th day, and S is the total number of seeds used. Mean shoot and root lengths at the end of germination were measured per replication. Dry weights of seedlings were taken with the help of an electric balance after drying each replication at 70°C in the oven to get the constant weight (Afzal *et al*., 2005). For statistical analysis, the data were transformed to arcsin X /100. Data were subjected to analysis of variance (ANOVA) procedures (SAS Institute Inc., 1988), and the LSD test was applied at a 5% probability level to compare the differences among treatment means.

3. Results and Discussion

The analysis of variance for the reduction percentages of germination (Tables 1 and 2), indicated a highly significant difference in interaction between seed priming treatments and osmotic potential in experiments with both NaCl and PEG (NaCl-induced salinity and PEG-induced drought). In both salinity and drought experiments, the germination percentage decreased significantly in accord with decreasing osmotic potential (Fig. 1a, b). This agrees with results reported by other studies, which have shown that the germination stage is the most sensitive stage of development by stress (Abbasdokht, 2010; Katembe, 1998). Hydroprimed and haloprimed seeds achieved minimum germination reduction percentages. Maximum germination reduction percentages were recorded for untreated seeds (control) (Fig. 1a, b). These results are in line with the findings of Thornton and Powell (1992) in Brassica and Srinivasan *et al*. (1999) in mustard. It is concluded that the superior effect of hydropriming on germination could be due to the effects of soaking time, because hydroprimed seeds compared to halopriming treated seeds were allowed to imbibe water for a longer time and went through the first stage of germination without protrusion of radicle. Akinola *et al*. (2000) reported that a higher duration of exposure to seed treatment resulted in higher cumulative germination in wild sunflower, and Caseiro *et al*. (2004) found that hydropriming was the most effective method for improving seed germination in the onion, especially when the seeds were hydrated for 96 h compared with 48 h. The beneficial effects of halopriming on germination were also seen in this study. In both seed priming treatments at different levels of osmotic potential, greater reductions in germination percentage were recorded for PEG than for NaCl (Fig.1a, b). Seeds always germinated better in NaCl than in PEG at equivalent water potentials, in line with earlier observations made for soybean by Khajeh-Hosseini *et al*. (2003).

This result may be due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination. The lower germination percentages obtained from PEG compared with those from NaCl at equivalent water potentials in each priming method suggest that the adverse effects of PEG on germination were caused by the osmotic effect rather than specific ion accumulation. These results agree with those of Murillo-Amador *et al*. (2002) in cowpea and Demir and Van De Venter (1999) in watermelon. They affirmed that drought or salinity may influence germination by decreasing water uptake. Priming may be helpful in reducing the risk of poor stand establishment under drought and salt stress and permitting more uniform growth under conditions of irregular rainfall and drought on saline soils. It was observed that hydropriming practically ensured rapid and uniform germination accompanied with low abnormal seedling percentage in line with Singh (1995) and Shivankar *et al*. (2003). They stressed that it has high potential in improving field emergence and ensuring early flowering and harvest under stress conditions, especially in dry areas. Our findings revealed that inhibition of germination at equivalent water potentials of NaCl and PEG resulted from the osmotic effect rather than salt toxicity. Both seed treatments gave better performances than the control (untreated) under salt and drought stresses, clearly demonstrating the effectiveness of hydropriming in improving germination percentages at low water potential.

Table 1. Factorial analysis of the seed priming effect on germination and seedling growth of corn under different levels of osmotic potential induced by NaCl (salinity)

Salinity Experiment	R.P.G.	R.P.L Root	R.P.L Shoot	R.P.D Root	R.P.D Shoot	Germination Index	Vigour Index
Seed Priming (a)	13.7*	11.5*	13.6*	8.9*	7.54*	62.1*	71.8**
Salinity (b)	23.9*	54.8**	15.9*	25.7*	14.32*	6.73*	35.4*
a*b	18.5*	63.5**	38.83**	38.9*	22.82*	31.33**	52.5*
Cv (%)	9.4	11.1	13.8	8.3	14.8	11.45	15.43

$^*P < 0.05$; $^{**}P < 0.01$;

RPG: reduction percentage of germination; RPL: reduction percentage of length; RPD: reduction percentage of dry weight

Table 2. Factorial analysis of the seed priming effect on germination and seedling growth of corn under different levels of osmotic potential induced by PEG (drought)

Drought Experiment	R.P.G.	R.P.L Root	R.P.L Shoot	R.P.D Root	R.P.D Shoot	Germination Index	Vigour Index
Seed Priming (a)	15.65*	35.2**	17.2*	18.1*	8.3*	18.5*	27*
Drought (b)	21.9*	17.8*	26.2*	62.2**	22.1*	30.8*	23.6*
a*b	11.73*	16.34*	13.2*	72.2**	56.1**	19.4*	14.2*
Cv (%)	14.6	17.4	8.5	15.1	11.02	15.2	9.05

$^*P < 0.05$; $^{**}P < 0.01$;

RPG: reduction percentage of germination; RPL: reduction percentage of length; RPD: reduction percentage of dry weight

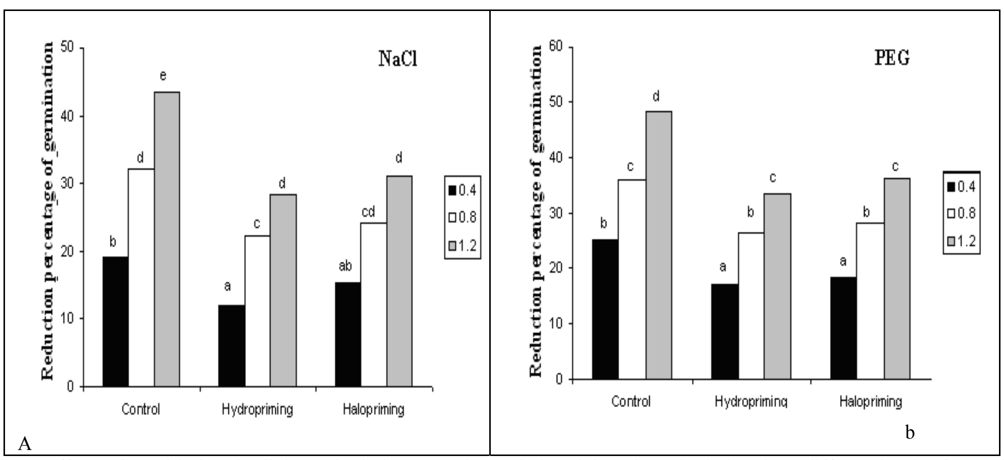

Fig. 1. Effects of NaCl-induced salinity and priming (a) and PEG-induced drought and priming (b) at different levels of osmotic potential on reduction percentage of germination (RPG) of corn

A significant interaction of seed priming×solution was found for the reduction percentages of root and shoot length (Table 1, 2). The minimum reduction percentage of root length (RPL root) was attained from hydroprimed and haloprimed seeds due to NaCl and PEG conditions (-0.4 MPa), and the maximum reduction percentage of root length was attained from controlled seeds due to NaCl and PEG (-1.2 MPa) conditions (Fig. 2a, b). There were significant differences among the controlled, hydroprimed, and haloprimed seeds for reduction percentage of root length due to NaCl and PEG conditions (Fig. 2a, b). The minimum reduction percentage of shoot length (RPL shoot) was attained from hydroprimed and haloprimed seeds due to NaCl and PEG (-0.4 MPa) conditions, and the maximum reduction percentage of shoot length was attained from the controlled seeds due to NaCl and PEG (-1.2

MPa) conditions (Fig. 2c, d). Murillo-Amador *et al*. (2002) found that seedling growth of cowpea was inhibited by both NaCl and PEG, but higher inhibition rates were caused by PEG. There were significant differences among the controlled, hydroprimed, and haloprimed seeds for reduction percentage of shoot length due to NaCl and PEG conditions at different levels of solution (Fig. 2c, d).

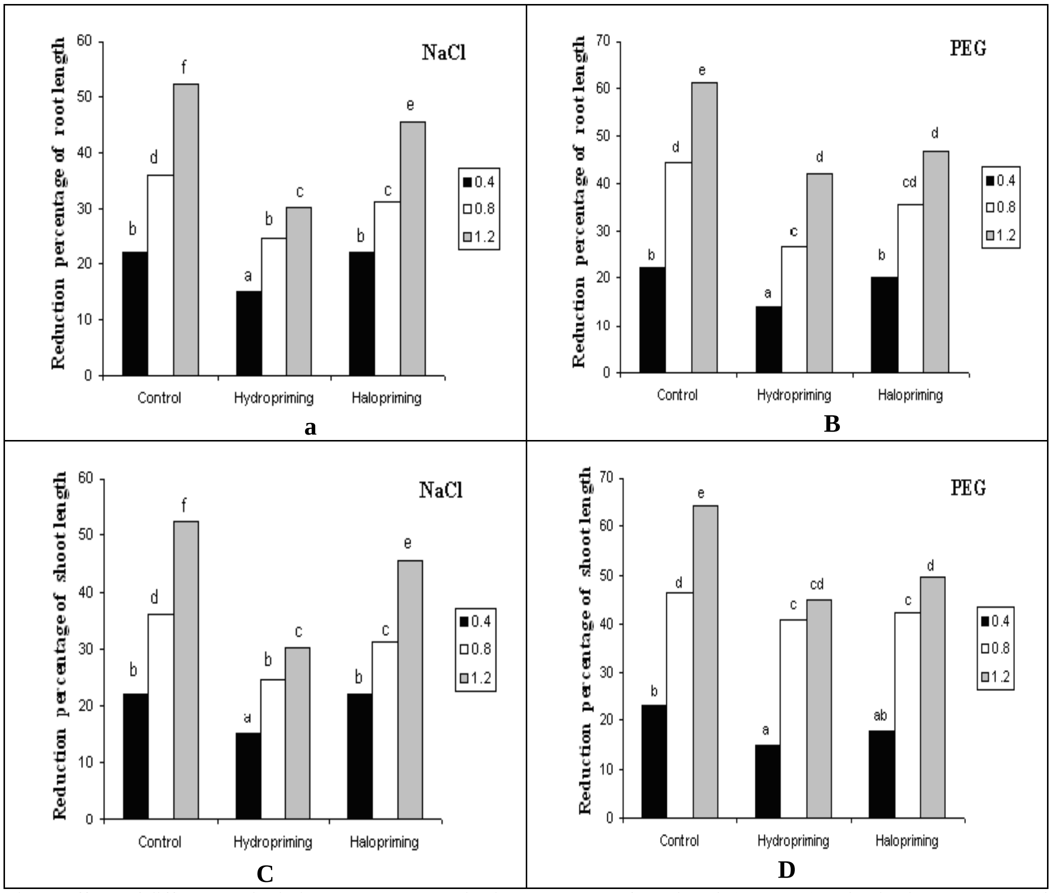

Fig. 2. Effects of different seed priming treatments on reduction percentage of root length (RPL root) and reduction percentage of shoot length (RPL shoot) of corn (*Zea mays* L.) from NaCl and PEG solutions under different osmotic potential levels

The reduction percentage of dry weight for root (RPD root) and reduction percentage of dry weight for shoot (RPD shoot) that were subjected to hydropriming was less than those subjected to halopriming and control, respectively.

Nematollahi *et al*. (2009) reported that hydropriming increased seedling dry weight under saline conditions. The RPD for root and shoot increased according to increases in osmotic potentials in both NaCl and PEG conditions (Fig. 3a, b, c, d). The interaction of seed priming treatment and osmotic potential for the germination index (GI) showed that under 0 and -0.4 MPa, hydroprimed and haloprimed seeds had higher GI than untreated seeds because of NaCl and PEG conditions (Fig. 4a, b). Interaction effects of seed priming treatment and osmotic potential significantly affected the vigour index (VI) due to NaCl and PEG conditions (Tables 1, 2), and hydropriming significantly increased VI at high (0, 0.4 MPa) osmotic potentials (Fig. 4c, d). The average VI of hydroprimed seeds was higher than that of untreated seeds at high osmotic potentials and was not significantly different with untreated seeds at low osmotic potentials (Fig. 4c, d). The present study has shown that both salinity and drought stress affected germination adversely. Compared with the control, both seed priming treatments showed enhanced performances under stress conditions.

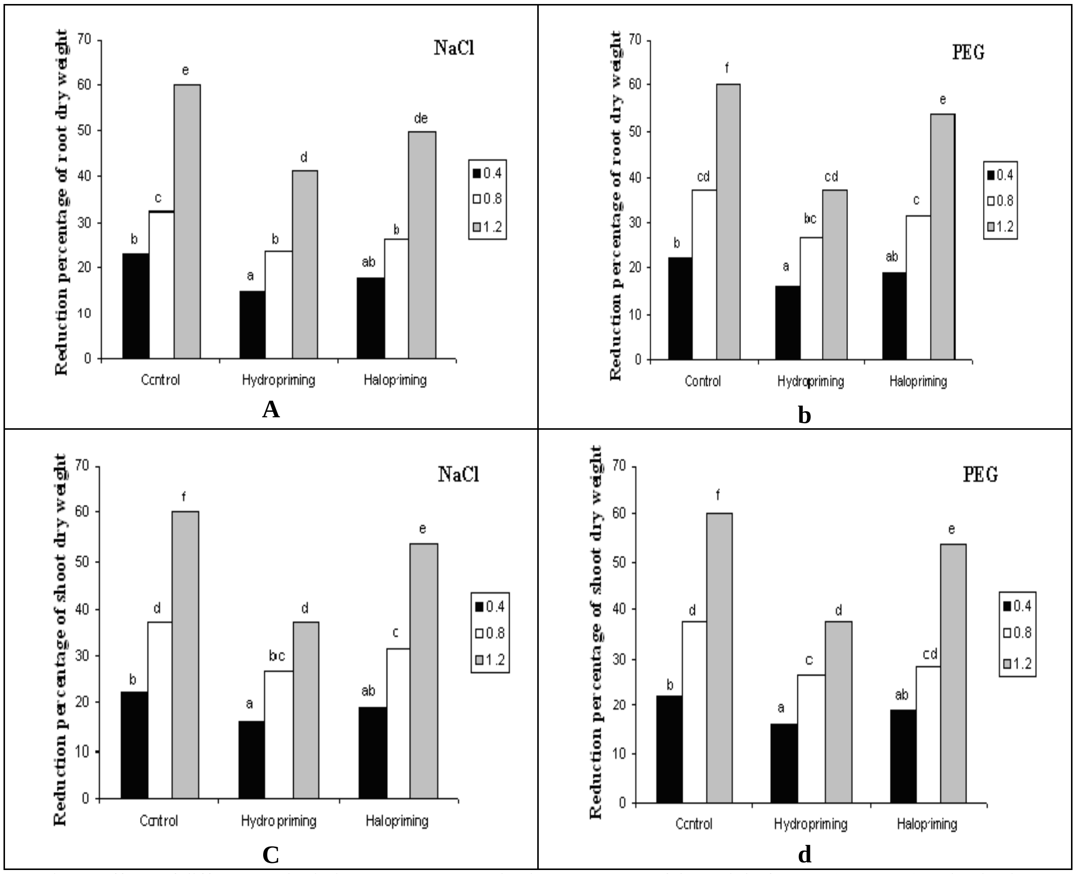

Fig. 3. Effects of different seed priming treatments on reduction percentage of dry weight for root (RPD root) and reduction percentage of dry weight for shoot (RPD shoot) of corn (*Zea mays* L.) from NaCl and PEG solutions under different osmotic potential levels

4. Conclusion

Hydropriming techniques and halopriming improved seed germination under both stress and non-stress conditions. Hydroprimed and haloprimed seeds achieved earlier and more uniform germination. The findings of the present study are in agreement with the results of Kaya *et al*. (2006), Harris *et al*. (1999), and Basra *et al*. (2006) who reported that hydroprimed seeds of sunflower and wheat could germinate faster and produce longer seedlings under salinity stress compared with untreated seeds. Some earlier studies reported that halopriming can contribute to improved germination rates and seedling emergence in different plant species by increasing the expression of aquaporins (Gao *et al*., 1999). Results of this study showed that halopriming with $CaCl_2$ and hydropriming can be recommended as suitable treatments under both stress and non-stress conditions. Hydroprimed seeds imbibed water for a longer time than haloprimed seeds and went through the first stage of germination without root protrusion. Seeds germinated better in NaCl than in PEG at equivalent water potentials, possibly due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination. With no toxicity caused PEG reported (Khajeh-Hosseini *et al*., 2003), the lower germination percentage obtained from PEG compared with NaCl suggests that adverse effects of PEG on germination were caused by osmotic effects rather than specific ion accumulation. Our results showed significant improvement in germination and early growth of corn (*Zea mays* L.) due to hydropriming and halopriming treatments. Soaking seeds for 32 h and halopriming with $CaCl_2$ resulted in better germination under salinity and drought stress. The findings of the present study also revealed that, at equivalent osmotic potentials, drought stress induced by PEG had more drastic inhibitory effects on germination. Thus, it is concluded that under salinity stress, the osmotic effect is rather more important than the toxic effect in loss of seed germination. It was observed that hydropriming practically ensured rapid and uniform germination accompanied with low abnormal seedling percentages in line with the results of Singh (1995) and Shivankar *et al*. (2003), especially when germination is affected by salinity and drought stress. Halopriming is a physiological method which improves seed performance and provides faster, synchronized germination. It has been shown that halopriming could be used as an adaptation method to improve the salt tolerance of seeds.

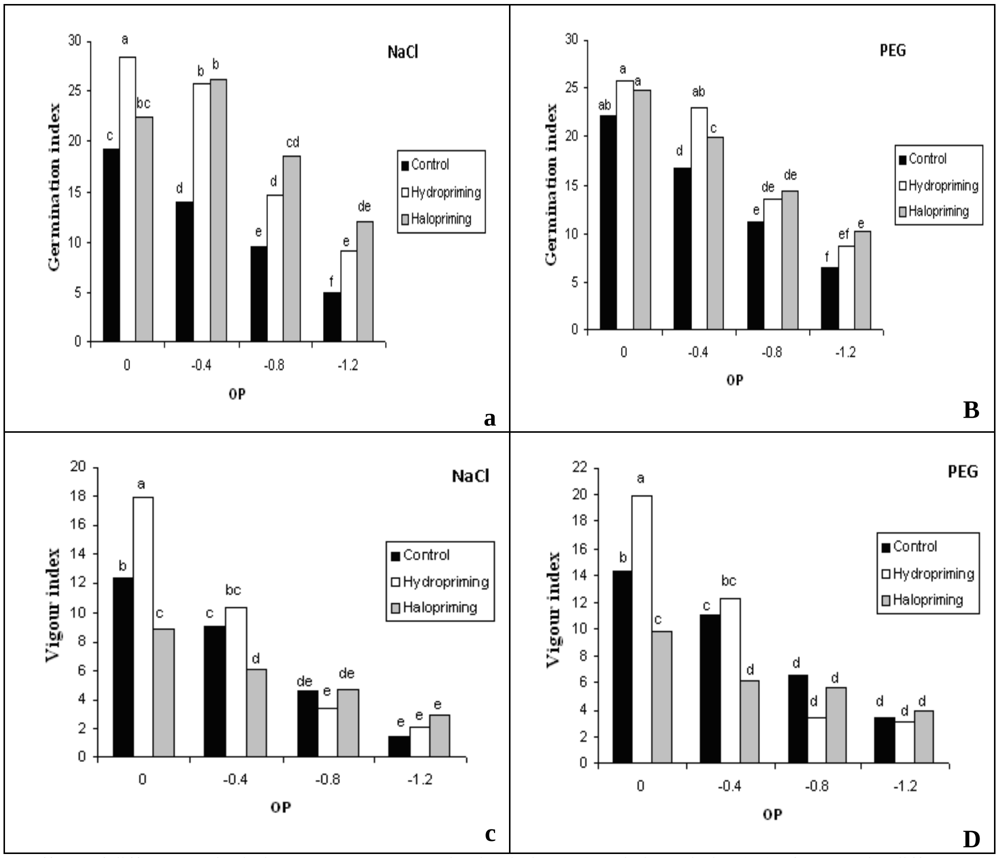

Fig. 4. Effects of different seed priming treatments on germination index (GI) and vigour index (VI) of corn under different osmotic potential (OP) levels. The bars marked with different letters of the alphabet are statistically different at P= 0.05. Germination index and vigour index of each osmotic potential are averaged from NaCl and PEG solutions)

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Crude Protein Content does not Determine the Preference Value of Plant Species for the Raini Goat (*Capra aegagrus hircus* L.) in Dry Rangelands

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Abstract

In order to estimate the relationship between forage quality and preference value of plant species for Raini goats (*Capra aegagrus hircus*), a field study was carried out on Raini goats' grazing behavior in some desert habitats. Crude protein (as the most important factor affecting forage quality) of all plant species was measured at two phenological stages (spring and summer, 2010) in the dry rangeland of Talkhabad, Iran. In addition, the preference value of plant species by free-ranging goats was estimated by visual estimation during the two seasons. One-way ANOVA and paired t-test revealed that forage quality and preference value by goats differed among plant species and between seasons. There was no significant relationship between forage quality and preference value (linear regression and Pearson correlation). Only a few plant species had both high quality and high preference (e.g., *Taverniera cuneifolia*). An interesting outcome was the importance of minor low-quality feeds such as *Ziziphus spina-christ* fruit in the goats' diet. Raini goats were also highly selective feeders, changing their diet from grazing to browsing and *vice versa*, which highlights the importance of diversified botanical structures and the preservation of shrubs and trees in their desert habitats.

Keywords: Dry rangeland; Forage quality; Raini goat; Plant phenological stage; Preference value

Nomenclature: Rechinger (1964)
Abbreviations: CP (Crude Protein), PI (Preference Index)

1. Introduction

The estimation of plant species' characteristics which affect their forage quality is necessary to improve the management of grazing regimes in rangelands. The relationship between herbivores and (a)biotic environmental factors is mutual. It has been repeatedly reported that herbivores directly influence the productivity, structure, and diversity of plant communities (Bakker, 1998; Adler *et al*., 2001; Vallentine, 2001; Loucougaray *et al*., 2004; Lamoot *et al*., 2005), forage quality of plant species (Erfanzadeh, 2009; Milotic *et al*., 2010) and soil physico-chemical characteristics (Sadeghi *et al*., 2005) through defoliation, trampling, production of excrement, and rolling. In previous studies, we demonstrated that ruminants can affect plant quality and diversity as well as the rate of some edaphic factors in different habitats (e.g., deserts and salt marshes; see Sadeghi *et al*., 2005 and Milotic *et al*., 2010, respectively). Nevertheless, the effects of vegetation characteristics on the behavior, diet selection, and performance of ruminants have not been well documented

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(Silveira Pontes *et al*., 2010), especially in desert habitats.

Understanding the diet selection and behavior of grazing animals in natural habitats, especially in fragile ecosystems, is important for an environmentally friendly management strategy and profitable animal production (Sanon *et al*., 2007). Moreover, optimal intake of nutrients by ruminants can be more easily achieved if we know their dietary habits and preferences (Ngwa *et al*., 2000). However, understanding food preferences is relatively difficult, since choices made by grazing or browsing herbivores of what and how much plant species to take are dependent on several factors, viz. local conditions (Field, 1979; Odo *et al*., 2001), spatial abundance of the preferred plant species or food (Dumont *et al*., 2002; Papachristou *et al*., 2007), phenological stage of the plant (Sanon *et al*., 2007), forage quality (Ball *et al*., 2000; Marquardt *et al*., 2010), and supplemental protein and energy (Dziba *et al*., 2007). Previous authors have repeatedly shown that there is a significant correlation between diet selection of large herbivores and crude proteins, i.e. protein alone often drives food choice (for steers: Hardison *et al*., 1954; for kangaroos: Tiver and Andrew, 1997; for white-tailed deer: Berteaux *et al*., 1998; for ewes: Silveira Pontes *et al*., 2010).

Among ruminants, goats are well-known for feeding upon a wide spectrum of plants and for possessing some degree of nutritional wisdom enabling them to select foods that meet their nutritional needs and avoid those causing toxicosis (Provenza *et al*., 1994a, b). Haenlein *et al*. (1992) reported that goats exhibit a definite preference for a varied diet, often consuming no less than twenty-five different plant species. Goats utilise a wide variety of plant types (Peter *et al*., 1979; Odo *et al*., 2001) and select from them the materials with the highest nutrient concentrations (Narjisse, 1991). However, in some cases, plants that constitute only a small proportion of a habitat make up a large part of the grazing animal`s diet (Ngwa *et al*., 2000). In addition, due to a wide temporal and spatial variation in forage preference and nutrient composition of diets selected by different types of animals, research findings from a given area and kind or class of animal have limited inferences and should not be applied to a wide area. It is therefore important to ascertain the locally available food types and preferred diets for a specific species and/or breed of livestock dominated in a particular region. Moreover, information on the food habits of pastoral animals is generally scarce; studies on the relationship between forage quality and diet preferences of goats are particularly missing for drylands. This field study, carried out in an Iranian desert, aimed to 1) estimate the crude protein content of plant species in dry rangelands. Because it has been proven that crude protein (further abbreviated as CP) content is the most important vegetation parameter that influences forage quality (Biondini, 1986), we considered only crude protein in our estimation of forage quality; 2) determine the preference value of plant species in Raini goats as the most important plant feeder in the study area; and finally, 3) find out if the time goats (*Capra aegagrus*) spend on grazing or browsing plant species is correlated to the quality of those plants.

2. Materials and Methods

2.1. Study area and sampling design

The study was carried out in the Talkhabad rangelands, a part of Kerman province, Iran (Fig. 1). The general climate of the study area (57º42´ to 57º46´ East; 28º02´ to 28º06´ North) is dry (domarton method), having 216.6 mm annual precipitation (Iranian Meteorological Organization: www.IRIMO.ir).

The vegetation is mostly xero-halophyte and largely dominated by annual species in spring and shrubs in summer (Table 1). Goat is the most important livestock that roam during the entire year, is corralled at night, and is let out by pastoralist and dog to feed during the day (Arzani, 2009). The studied goat (*Capra aegagrus hircus*), natively named Raini, is the most important cashmere variant in Iran, and it has great economic wealth. This breed is encountered in the southeast of Iran, in Kerman province (Sakha *et al*., 2009).

Diet selection and vegetation data were collected in 2010 during two seasons: in spring, from 15 to 30 March, when annual plant species dominate the vegetation, and in summer, from 23 July to 6 August, when perennial species are dominant. A preliminary survey was done at the beginning of both sampling periods in order to identify all plant species present in the study area. Despite the domination of shrubs in parts of the area during the last period, shrub individuals were mostly sparse. Forty 1*1 m random plots were used to estimate relative cover of all plant species during each period. Above-ground vegetation composition was determined during the two periods (vegetative growth stage and seed ripening stage) by estimating the cover of all plant species (according to the method described in Londo, 1976).

2.2. Vegetation data

At each plot, the above-ground biomass of herbaceous and current-year growth of woody species (with the exception of *Ziziphus spina-christi*) was cut using garden shears, leaving a stubble height of approximately 1 cm for herbaceous species. As the twigs of *Ziziphus spina-christi* were not available for browsing, only the fruit fallen onto the ground were collected during the spring sampling. In summer, the fruit of that species was not observed. Herbage samples were hand-sorted in the laboratory into several subsamples at the species level, and the subsamples were subsequently dried at 65ºC for 48 h to a constant weight (Milotic *et al*., 2010). Next, the protein content of each plant species was measured using the Kjeldahl crude protein method (AOAC, 1984).

Fig. 1. Location of the study area in Talkhabad, Kerman province (Iran)

Table 1. List of plant species in the study area (A= annual, P= perennial)

Species	Life form	Family
Asphodelus tenuifolius Wendelbo	Aherb	*Liliaceae*
Astragalus triboloides Delile	A forb	*Leguminoseae*
Calligonum bungei Rech. & Sch.-Cze	P-small tree	*Polygonaceae*
Fagonia bruguieri Hadidi	A forb	*Zygophyllaceae*
Gaillonia aucheri Guill	P-shrub	*Rubiaceae*
Gymnocarpus decander Forssk	P-cushion	*Caryophyllaceae*
Hammada salicornicum (Moq.) Bunge ex Boiss.	P-shrub	*Chenopodiacese*
Lycium edgeworthii Sch.-Tem.	P-small tree	*Solanaceae*
Plantago stocksii Rech.	A forb	*Plantaginaceae*
Rhazya stricta Dc.	P-shrub	*Polygonaceae*
Stipa capensis Thunb.	A herb	*Gramineae*
Taverniera cuneifolia (Roth) Arn.	P-shrub	*Fabaceae*
Ziziphus spina-christi Boiss.	P-tree	*Rhamnaceae*
Zygophyllum eurypterum Hadidi	P-shrub	*Zygophyllaceae*

2.3. Diet selection data

The method used to observe forage preference involved close observation of one randomly selected focal adult-female goat grazing with the others in the flock (Odo *et al*., 2001). Each focal goat was closely monitored by one observer who recorded the time spent on each plant species when the goat grazed or browsed. Observations were made during three hours (8am to 11am) on five different days for each period. Preference indices were then estimated for all plant species with the assumption that the time spent on a plant reflects the proportion of that plant in the diet (Becker and Lohrmann, 1992). From these records (five randomly-selected goats during five days; each day one goat was observed by the same observer), the following parameters were calculated: relative cover of the plant species in the habitat and proportion of individual plant species in the diet for each record (each day comprised three hours) and the preference index (further abbreviated as PI), measured as (Ngwa *et al*., 2000):

$$\text{Preference Index (PI)} = \frac{\text{Proportion of plant consumed}}{\text{Proportion available in the range}}$$

$$= \frac{\text{Time spent feeding on the plant species expressed as percentage of total feeding time}}{\text{Relative cover of plant species in the habitat}}$$

Preference indices were calculated for all species, for each record (5 days), and for both sampling periods.

2.4. Data analysis

As the data met normal distribution requirements, parametric analyses were performed using SPSS version 17. First, ANOVA and post-hoc (Duncan) tests were used to compare forage quality and PI among species for both periods separately. Then, the results obtained from forage quality (and PI) collected during spring and summer were treated likewise using paired t-test. Finally, linear inter-regression and Pearson correlation methods were used to estimate the relationship between forage quality and PI.

3. Results

Fourteen plant species were identified during the two sampling seasons (Table 1). Crude protein content was significantly different among species and between two phenological stages. Preferences of plant species for grazing by goats were also significantly different among plant species and between the two sampling periods.

During spring (vegetative growth stage), the highest crude protein content was recorded in *Astragalus triboloide*s with 19.6%, while *Ziziphus spina-christi* had the lowest CP content with 4.22% (Table 2). The highest preference index was found in *Plantago stocksii* with an average index of 3.24, while the lowest index was in *Asphodelus tenuifolius*, *Rhazya stricta* and *Hammada salicornicum* with an average value of 0.00. The latter plant species occurred in the study area, but were never grazed by goats (Fig. 2). During summer (seed ripening stage), the crude protein content was highest in *Rhazya stricta* with 14.85% and lowest in *Stipa capensi*s with 5.23%. The highest preference index was recorded in S*tipa capensis* with an average index of 7.37, while the preference value of *Rhazya stricta*, *Lycium edgeworthii,* and *Calligonum bungei* was 0.00 (Fig. 3). There was no significant correlation between CP (%) and PI for both sampling seasons (Table 3). The crude protein of species significantly decreased between spring and summer (from vegetative growth to seed ripening stage), whereas the preference index was higher in summer compared to spring (Table 4).

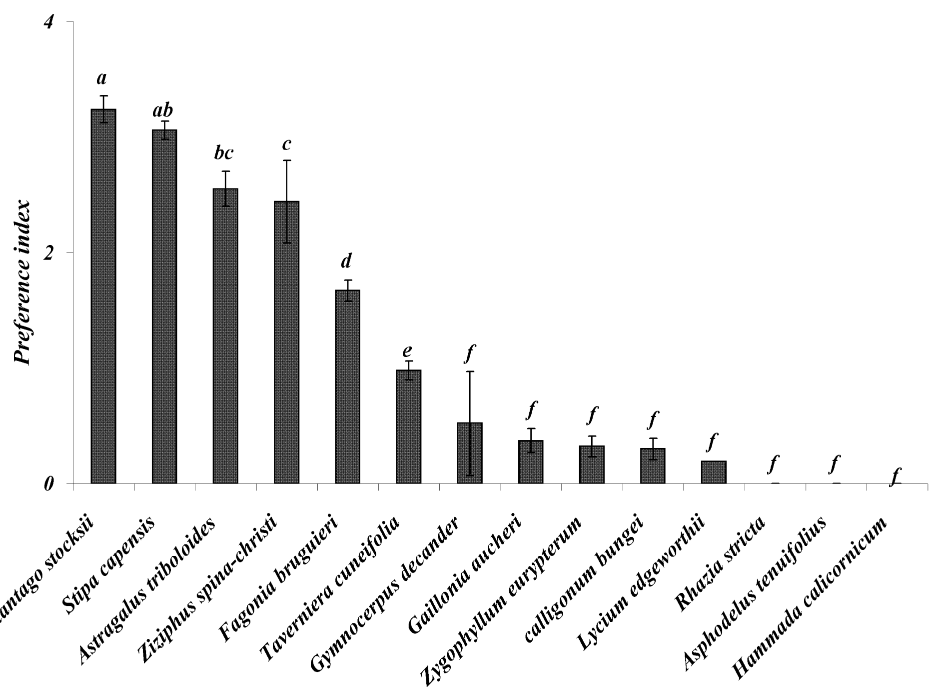

Fig. 2. Preference indices of plant species (Mean ± SE) during the first sampling session (spring 2010) and significance by ANOVA followed by post-hoc (Duncan) tests (different successive letters indicate significant differences at P < 0.05)

Table 2. Average (±SD) of crude protein content (CP, %, and cover percentages of the different plant species during the two sampling periods (n= 40 samples per plant and season)

Species	CP in spring	CP in summer
Asphodelus tenuifolius	15.97±1.02	5.59±1.13
Astragalus triboloides	19.60±0.82	8.52±0.41
Calligonum bungei	7.79±0.67	5.84±0.00
Fagonia bruguieri	16.48±0.70	10.45±1.58
Gaillonia aucheri	7.54±0.65	9.87±0.79
Gymnocarpus decander	9.42±1.49	6.01±1.08
Hammada salicornicum	10.99±0.42	13.41±3.13
Lycium edgeworthii	7.90±0.62	6.74±0.82
Plantago stocksii	11.85±1.02	5.90±2.01
Rhazya stricta	14.58±0.82	14.85±0.63
Stipa capensis	10.95±0.76	5.23±0.12
Taverniera cuneifolia	12.28±1.45	11.29±2.77
Ziziphus spina-christi	4.22±0.40	-
Zygophyllum eurypterum	13.33±0.81	9.00±0.00

Table 3. Relationship between crude protein content and preference indices using inter-regression and Pearson`s correlation methods (ns: not significant)

Sampling session		Spring	Summer
Inter-regression	t	0.94	0.51
	Sig. (p-value)	0.333 (ns)	0.622 (ns)
	B	0.53	0.147
Pearson`s correlation	R	0.141	-0.158
	Sig. (p-value)	0.935 (ns)	0.625 (ns)

Table 4. Comparison of crude protein content and preference index between spring and summer and significance by paired t-tests (*P < 0.05)

	Preference index	Crude protein (%)
Mean in spring ± SD	1.22 ± 1.40	11.64 ± 4.11
Mean in summer ± SD	2.75 ± 2.99	9.53 ± 3.31
t	2.21	2.63
Sig. (p-value)	0.044 (*)	0.022 (*)
df	11	12

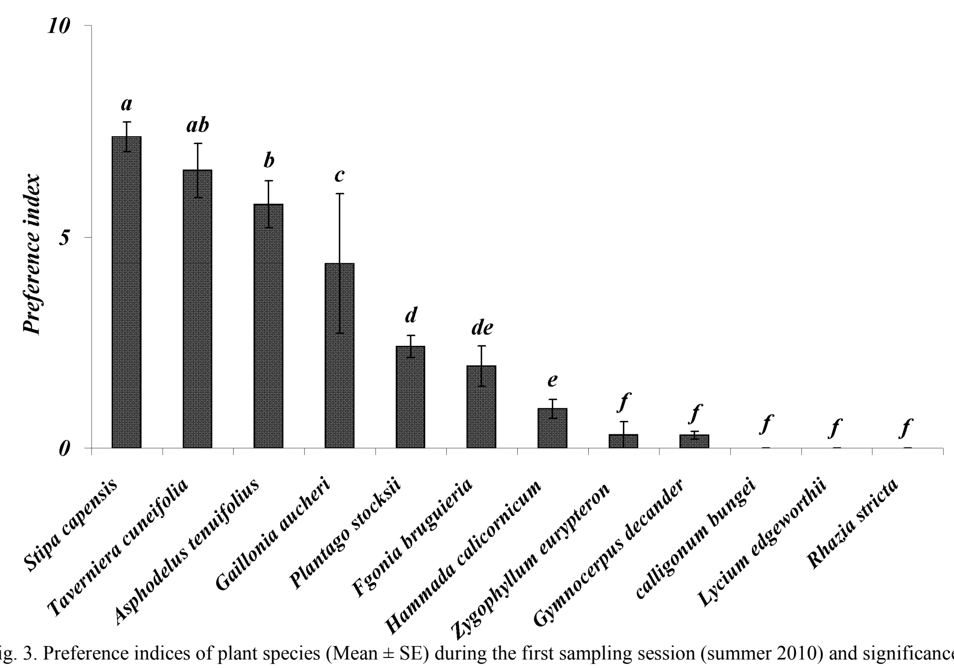

Fig. 3. Preference indices of plant species (Mean ± SE) during the first sampling session (summer 2010) and significance by ANOVA followed by post-hoc (Duncan) tests (different successive letters indicate significant differences at P < 0.05). During summer, the fruit of *Ziziphus spina-christi* was not observed, and the preference index of *Astragalus triboloides* was not measurable because it occurred veryrarely

4. Discussion

Recognizing the factors that influence diet selection was a hard task in this study. Predicting a grazing animal`s diet is actually difficult because of variations among animal species, and due to the location, weather, and maturity and availability of plants (Malechek, 1983; Cosyns *et al*., 2001; Lamoot *et al*., 2005). Hence, a more general characteristic is needed to represent diet selection. A good candidate might yet be crude protein of plant which was investigated in this research. However, in this study, plant species, besides having different qualities, were not selected according to their forage qualities. Crude protein in annual species varied within two phenological stages and declined from spring to summer. A higher CP was observed during spring when annual species were mostly in a vegetative growth. Decreased forage quality is expected as plants mature and leaves senesce (Angell *et al*., 1990; Ganguli *et al*. 2010). For most perennial species, this decrease in CP was less pronounced between the two phenological stages, and crude protein remained constant or increased slightly between the two sampling seasons. The nutritive value of browse species is known to be high, with low variation over time compared to grasses (Fadel Elseed *et al*., 2002). Moreover, crude protein significantly differed among plant species. Different factors affect forage quality, and the kind of species is indeed one of the most important factors influencing the rate of forage quality (Lyons *et al*., 1994; Chen *et al*., 2001).

Contrary to our expectations, plant species were not selected according to their nutritive qualities (CP). During spring, *Asphodelus tenuifolius* with 15.97% CP, *Rhazya stricta* with 14.58% CP, and *Hammada salicornicum* with 11% CP all had relatively high quality. Nevertheless, they were not grazed (or browsed) by goats. In addition, *Rhazya stricta* with the highest CP from the summer values were not grazed by goats. Therefore, it can be concluded that some other plant traits determine whether a plant species will be eaten or not. Indices of forage quality (CP) were not significantly correlated with selectivity, conforming previous findings from Ganskopp *et al*., (1996) and Alonso-Diaz *et al*., (2008). Anatomical features such as thorns, awns, and dense pubescence can affect a plant`s preference value (Malechek and Provenza, 1983). Garin (1997) demonstrated that browsing on some woody species was very light. In our study area, those woody legumes had hard prickles between their protein-rich leaves, and browsing on those species was constrained mainly by the morphological features (spiny cushion form) rather than by their nutritive value. In addition, secondary metabolites lead ruminants to limit their intake of even the most nutritive food (Tanner *et al*., 1990), which in turn leads them to ingest a diverse array of plants to minimize toxicosis (Ngwa *et al*., 2000). Particularly, the preference of goats for woody and herbaceous plant species, in spite of lower intake rate of biomass and nitrogen, may be governed by the need of goats to avoid high levels of phenolics and tannins more than to maximize intake of nutrients and energy (Woodward and Coppock, 1995). Cooper and Owen-Smith (1985) found preferences of goats for woody plants to be little related to nutrient concentration, but to be low when concentration of phenolics exceeded 5%. The results of this study are contrary to the results of few authors (Aregheore *et al*., 2006; Johansson *et al*., 2010). The latter studies reported that there was a significantly positive (or negative) correlation between CP and selectivity of plant species by goat. In addition, Ngwa *et al*. (2000) demonstrated that goats are more selective feeders than sheep and cattle, tending to select the better quality (higher protein) plant species.

Surprisingly, *Stipa capensis* was a good candidate to be eaten by the goats. *Stipa capensis* Thunb. (Poaceae) is common as an annual grass in dry habitats around the Mediterranean and the Middle East up to the Persian Gulf. It is distributed in large areas of the Irano-Turanian phytogeographic regions (Boeken *et al*., 2004). This species has seeds with erect appendages which cause serious problems for large herbivores, and it is always reduced through management measures (Arzani, 2009). However, this species was a major part of the goats' diet in both sampling sessions, while it had a relatively low CP. During the spring sampling, *Stipa capensis* was in its vegetative stage and had soft and succulent tissues. In summer, the seeds of that species have already been dispersed, and the plant has dry but soft tissues. Therefore, the soft tissues of *Stipa capensis* might attract goats, inducing a relatively high preference index. *Ziziphus spina-christ* showed a high preference value during spring. We observed that goats ignored other neighboring species when they were confronted with that species. The goats browsed only on the fruit that had fallen to the ground. Previous studies also have reported that goats were more interested in the blossoms and pods of certain browse species, e.g., *Guirra senegalensis* and

Combretum micrantum (Ngwa *et al*., 2000). We also observed that the goats were not able to browse the twigs and leaves of *Ziziphus spina-christ* due to the high height of this plant species, emphasizing again the importance of plant availability in grazing or browsing (Odo *et al*., 2001; Goetsch *et al*., 2010; Marquardt *et al*., 2010).

Lycium edgeworthii was browsed in spring, yet it was not considered in the summer. The massive number of its hard prickles in the summer might prevent goats from browsing on this species. Spiny plant species may be less selected by ruminants due to morphological problems (Garin, 1997). In contrast, some species were selected more in summer than in spring (e.g., *Hammada salicornicum*). The production of secondary compounds during spring might influence these selections (Ball *et al*., 2000). Some species (e.g., *Taverniera cuneifolia*) showed both a relatively high quality and a high preference value. Although plant defences are abundant in wooded rangelands, they are not a complete barrier to small ruminants who often use woody plants as part of their diets (Papachristou *et al*., 2005), which indicates the importance of woody plants in rangelands as part of the diet of small ruminants.

Crude protein of dry rangeland plants is an unreliable index for predicting diet selection by goats. Further studies should investigate the role of other parameters, such as fibers and toxin or mineral concentration, in the preference value of plant species for Raini goats in dry rangelands. An interesting outcome is the importance of minor feeds such as fruits in a goat's diet. Range management measures, therefore, have to consider this opportunistic feeding, especially by maintaining some highly sparse plant species. Goats are highly selective feeders, changing their diet from grazing to browsing and *vice versa*, which highlights the importance of a diversified botanical structure and the preservation of shrubs and trees in desert habitats. Forage quality of plant species and their preferences for grazing are drastically affected by age; thus, further attention should be paid to plant species that have high both quality and preference for a longer time span (e.g., *Taverniera cuneifolia*). Rangeland management programs need to favor such valuable species, especially during the summer when these species are more prominent and the annual species have already dried.

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