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EFFICACY OF OSMOPRIMING ON EMERGENCE POTENTIAL AND SEEDLING VIGOUR OF RANGELAND GRASSES IN PAKISTAN

IHSAN QADIR[1*], MUHAMMAD FAROOQ AZHAR[1], MUHAMMAD YOUSAF ALI[1], KHAYYAM ANJUM[2] AND KARIM YAR ABBASI[3],

[1]*Department of Forestry & Range Management, Bahauddin Zakariya University, Multan-Pakistan*
[2]*Department of Forestry & Range Management, University of Agriculture Faisalabad-Pakistan*
[3]*Institute of Horticultural Sciences, University of Agriculture Faisalabad-Pakistan*
[*]*Corresponding author's email: mrbhabha@gmail.com*

Abstract

Rangelands of Pakistan have poor vegetation cover mainly due to very less emergence rate and poor growth behaviour of rangeland grasses. In this study Osmopriming techniques for improving emergence, growth behavior and biomass production of highly nutritive and palatable rangeland grasses were tested. For this, two rangeland grasses i.e., *Cenchrus ciliaris* and *Panicumantidotale* were selected and osmoprimed for 24 h with PEG-8000 solutions of varying osmotic potentials (-0.2, -0.6, -1.2 and -2.4 MPa). It was observed that *Cenchrus ciliaris* and *Panicum antidotale* seeds emergence was improved when primed at -0.6 and -1.2 osmotic potential PEG-8000 solutions. Maximum shoot length of *C. ciliaris* was found in osmoprimed seeds (-1.2 MPa) while root length was observed when the seeds were subjected with PEG-8000 (-2.4 MPa) solution while maximum root length of *P. antidotale* was recorded when seeds were primed with PEG-8000 (-1.2 MPa) solution. *C. ciliaris* and *P. antidotale* plants exhibited maximum dry weight when primed with PEG-8000 solutions (-1.2 and -0.6 MPa, respectively). It can be safely concluded from findings that the rate of emergence, final emergence percentage and biomass production of these two rangeland grasses can be improved by subjecting their seeds with PEG-8000 solutions at low osmotic potential ranging between -0.6 and -1.2. The findings of this study will help in improving the vegetation cover of rangelands in Pakistan.

Key words: *Cenchrus ciliaris*, Emergence, growth, Osmopriming, *Panicum antidotale*, PEG-8000, Rangeland.

Introduction

Pakistan has a diver climatic conditions ranging from coastal areas to alpine mountains including irrigated plains and rangelands. Rangelands are significant part of our national assets which can never be neglected. About 27% livestock heads mainly depend on rangeland grasses and forages but the rangelands of Punjab, Khyber Pakhton Khaw and Azad Jammu Kashmir provide only 30-40% livestock requirements of total livestock heads while Sindh and Balochistan cover 60 and over 80% requirements, respectively (Quraishi *et al.*, 1993). Poor germination seed and less plant cover are the main problems of rangelands in Pakistan.

Pakistan natural flora contains a few very good perennial forage grasses which need to be propagated through artificial reseeding and stump planting (Qamar *et al.*, 2000). Near absence of suitable winter growing grasses is a serious draw-back of our vast subtropical rangeland areas while most of these grasses are summer grasses which lack water availability in the required season. On the other hand there are many factors of varied nature responsible for low range production like drought coupled with very high and low temperature and strong winds, light textured soil of low fertility with low organic contents and centuries long misuse and vegetation exploitation in shape of over grazing and deforestation. The productivity of rangelands of Pakistan has declined up to 50 to 10% of its potential. Whereas the productivity of Thal range area is also very low due to drought, salinity, low soil fertility and low organic matter and light texture of the soil. On the other hand, range grasses of this area have poor germination rate and low productivity. This germination and plant vigor problems can be recovered by using the techniques of seed priming.

Seed priming is a suitable pre-sowing seed treatment in which seeds are held at low osmotic potential to imbibe preventing the radical protrusion (Bradford, 1986). Different seed priming techniques like Hydropriming, osmopriming, matripriming, hormonal priming etc. showed good promising results on different crops and vegetables (Basra *et al.*, 2002, 2004 & 2005, Farooq *et al.*, 2006a and 2008). In priming of range grasses' seeds, mostly matripriming and osmopriming with PEG solution have been used to increase germination rate and biomass production (Hardegree, 1996, Qadir *et al.*, 2011) but previously no work has been undertaken on *Cenchrus ciliaris*and *Panicum antidotale* in Pakistan. Both species *C. ciliaris* and *P. antidotale* are high valued nutritional and palatable range grasses for livestock. Due to importance of these species as fodder grass they were selected to evaluate seed germination and plant vigor improvement as effected by osmopriming ultimately to improve the condition of rangelands.

Materials and Methods

Seed collection: Seeds of two range grasses i.e., *Cenchrus ciliaris* and *Panicum antidotale* were collected from Punjab Forest Research Institute (PFRI) Faisalabad, Pakistan. Pretreatment germination percentage was found at 39.3 and 31.7% in *C. ciliaris* and *P. antidotale*, respectively.

Seed treatments: Seeds of both grasses were divided into 6 seed lots containing 25 seeds in each. Four lots were osmoprimed with PEG-8000 at osmotic potentials (-0.2, -0.6, -1.2 and -2.4 MPa) for 24 h at 27±2ºC. One seed lot was primed with distilled water (hydropriming) (Farooq*et* *al*., 2006b) for same duration while one seed lot was used as control which was unprimed. PEG-8000 solutions were prepared by using Michel (1983) protocol. During priming, continuous air was applied to solutions. After priming, osmoprimed seeds were washed and rinsed with distilled water followed by shade drying till their original weight. The seeds were packed in air tight bags till further use.

Seed emergence and vigour testing: Osmoprimed, hydroprimed and unprimed seeds were sown in earthen pots (9 inches in diameter) filled with sand, silt and clay (1:1:1) in wire-house under temperature ranging between 34-37ºC with 16 h daylight and 8 h night period. The experiment was conducted in completely randomized design (CRD) with three replications. Water was applied after every two days and no fertilizer was applied.

Seed emergence was observed daily and emerged seeds were counted according to the rule of testing feed Anon., (1990). The time taken for 50% of seed emergence of (E50) was calculated according to Farooq *et al*., (2005) equation and mean emergence time (MET) was calculated according to equation of Ellis &Roberts (1981). While emergence index (EI) was calculated following the formula given by Anon., (1983) and final emergence was expressed in percentage. Following equations were used for computing E50, MET and EI.

$$E50 = ti + \frac{(\frac{N}{2} - ni)(tj - ti)}{nj - ni}$$

Where N is the final number of seeds emerged and n_i, n_j cumulative number of seeds emerged by adjacent counts at times ti and tj when ni< N/2 <nj.

$$MET = \frac{\sum Dn}{\sum n}$$

where n is the number of seeds emerged on day D (D is the number of days counted from the beginning of emergence.)

$$EI = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + - - - - - - - + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

After final emergence, three plants in each pot were maintained for investigating growth parameters. These plants were harvested when reached 25 d age and shoot and root lengths, plant fresh and dry weights and tillers were recorded according to standard procedures.

Statistical analysis: The experiment was conducted two times for the reliability of data. Data was pooled, computed and analyzed by using a Computer Program MSTAT C (MSTAT Development Team, 1989). Difference in means was computed through LSD test at 5% level of probability (Steel *et al*., 1997).

Results and Discussion

Emergence phase: Emergence percentage and speed was significantly affected by osmopriming techniques. Maximum FEP (82.67%) in *C. ciliaris* seeds was found when those were subjected to PEG-8000 (-0.6 MPa) solution followed by -2.4 and -1.2 MPa osmotic potential (OP) solutions (60.00 and 57.33%) while unprimed seeds showed only 33.33% FEP (Table 1). In case of *P. antidotale*, PEG-8000 (-1.2 MPa) primed seeds exhibited maximum FEP (84.00%) which was statistically at par with PEG-8000 (-2.4 MPa) (84.00%) followed by -0.2 MPa OP (77.33%) while 49.33% FEP was calculated in unprimed seeds which was minimum among all other priming treatments (Table 1). In case of emergence index (EI), PEG-8000 (-0.6 MPa) primed *C. ciliaris* seeds showed maximum EIvalue (18.35) followed by PEG-8000 (-2.4 MPa) (15.28) while hydroprimed and unprimed seeds showed 6.64 and 4.39 EI, respectively which were statistically similar (Table 2). *P. antidotlale* seeds showed maximum EI (23.87) when primed with PEG-8000 (-2.4 MPa) followed by PEG-8000 (-1.2 MPa) (21.63) while unprimed *P. antidotale* seeds showed 8.32 EI value which was statistically at par with hydropriming (10.52) (Table 2). Minimum time taken for 50% emergence (E50) was observed when seeds of both grasses i.e., *C ciliaris* (5.53 d) and *P. antidotale* (5.84 d) were primed with PEG-8000 (-2.4 MPa) while unprimed seeds took 12 and 10.69 d for 50% emergence, respectively which was statistically at par with hydroprimed *P. antidotale* seeds (10.92 d) (Table 1).Moreover, all priming treatments improved mean emergence time (MET) of *C. ciliaris* seeds as compared to unprimed seeds but no significant improvement in MET was found in *P. antidotale* seeds. Minimum MET by *C. ciliaris* seeds (15.25 d) was recorded when seeds were primed with PEG-8000 (-2.4 MPa) solution while unprimed and PEG-8000 (-1.2 MPa) primed seeds were statistically similar with each other (Table 2).

Table 1. Effect of PEG-8000 osmopriming on final emergence percentage (FEP) and emergence index (EI) of *C. ciliaris* and *P. antidotale*.

Treatments	FEP		EI	
	C. ciliaris	***P. antidotale***	***C. ciliaris***	***P. antidotale***
Control	33.33 ± 2.16 d	49.33 ± 3.27 d	4.39 ± 0.57 c	8.32 ± 0.45 c
Hydropriming	38.67 ± 1.63 cd	65.33 ± 1.63 c	6.64 ± 0.31 c	10.52 ± 0.91 c
PEG-8000 (-0.2 MPa)	48.00 ± 2.83 bc	77.33 ± 3.49 b	8.49 ± 0.36 bc	17.58 ± 0.46 b
PEG-8000 (-0.6 MPa)	82.67 ± 2.94 a	76.00 ± 2.82 b	18.36 ± 0.35 a	17.26 ± 0.59 b
PEG-8000 (-1.2 MPa)	57.33 ± 1.63 b	84.00 ± 2.55 a	11.70 ± 0.17 abc	21.63 ± 0.72 b
PEG-8000 (-2.4 MPa)	60.00 ± 2.83 b	84.00 ± 2.12 a	15.28 ± 0.73 ab	23.86 ± 0.28 a
LSD 5%	13.10	6.50	7.76	4.57

Means showing different letters are not statistically similar (p <0.05) with each other

Table 2. Effect of PEG-8000 osmopriming on time taken for 50% emergence (E50) and mean emergence time (MET) of *C. ciliaris* and *P. antidotale*.

Treatments	MET		E50	
	C. ciliaris	*P. antidotale*	*C. ciliaris*	*P. antidotale*
Control	16.32 ± 0.02 a	15.35 ± 0.03	12.00 ± 0.35 a	10.69 ± 0.35 a
Hydropriming	15.32 ± 0.06 b	15.78 ± 0.06	9.67 ± 0.41 ab	10.92 ± 0.10 a
PEG-8000 (-0.2 MPa)	15.77 ± 0.09 ab	15.34 ± 0.03	7.56 ± 0.36 b	7.93 ± 0.37 b
PEG-8000 (-0.6 MPa)	15.77 ± 0.07 ab	15.38 ± 0.06	7.73 ± 0.52 b	8.67 ± 0.54 b
PEG-8000 (-1.2 MPa)	15.89 ± 0.07 a	15.12 ± 0.02	6.97 ± 0.39 b	7.22 ± 0.38 bc
PEG-8000 (-2.4 MPa)	15.25 ± 0.08 b	15.46 ± 0.02	5.53 ± 0.38 b	5.84 ± 0.42 c
LSD 5%	0.56		4.25	1.70

Means showing different letters are not statistically similar ($p < 0.05$) with each other

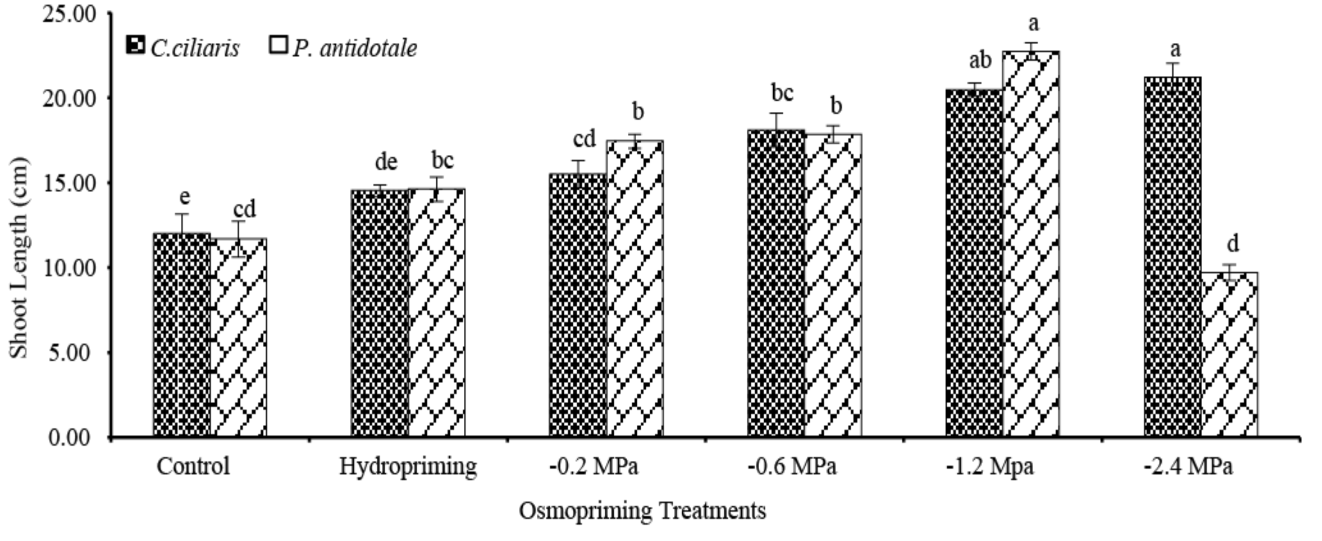

Fig. 1. Effect of PEG-8000 osmopriming on shoot length (cm) of *C. ciliaris* and *P. antidotale*. Means showing different letters are not statistically similar ($p<0.05$) with each other. ± vertical bars represent standard errors. Data were computed from three replications consisting of three plants in each replication.

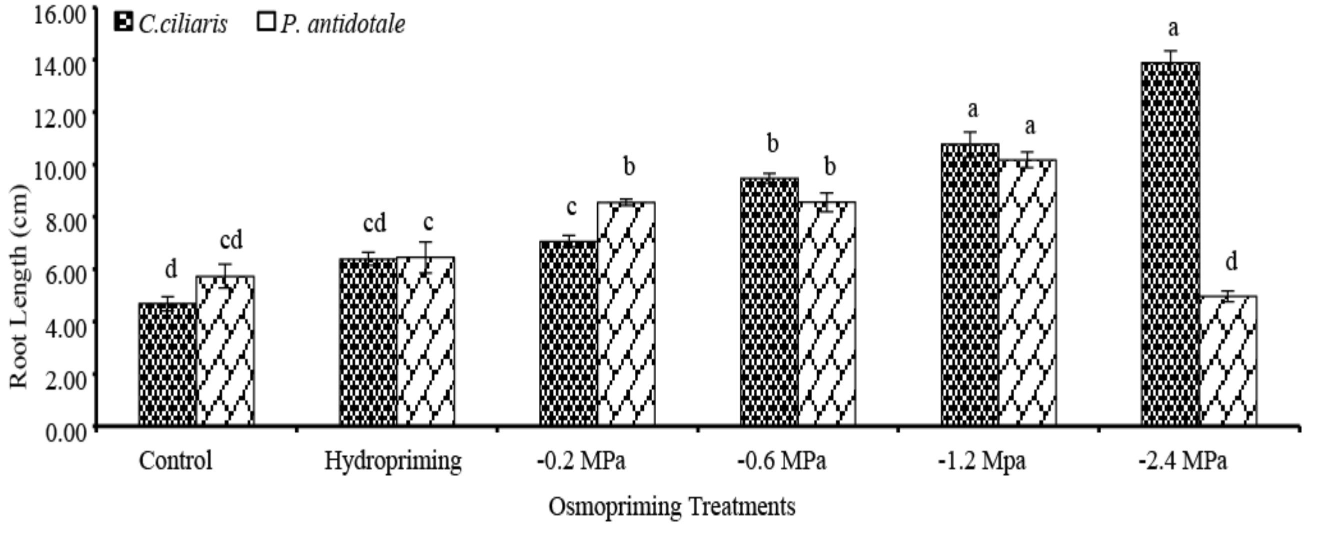

Fig. 2. Effect of PEG-8000 osmopriming on root length (cm) of *C. ciliaris* and *P. antidotale*. Means showing different letters are not statistically similar ($p<0.05$) with each other. ± vertical bars represent standard errors. Data were computed from three replications consisting of three plants in each replication

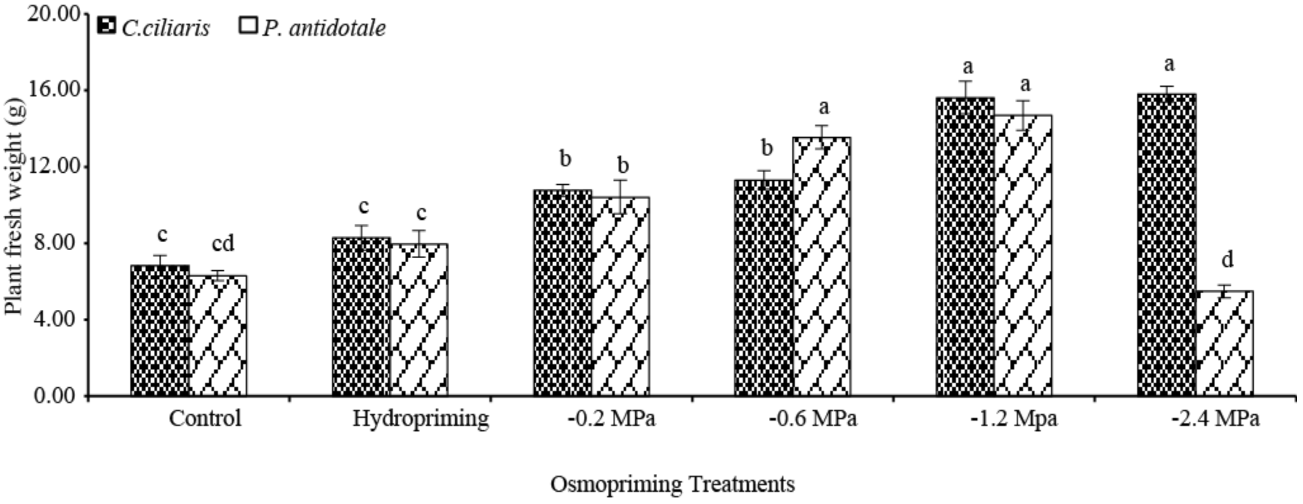

Fig. 3. Effect of PEG-8000 osmopriming on plant fresh weight (g) of *C. ciliaris* and *P. antidotale*. Means showing different letters are not statistically similar ($p<0.05$) with each other. ± vertical bars represent standard errors. Data were computed from three replications consisting of three plants in each replication

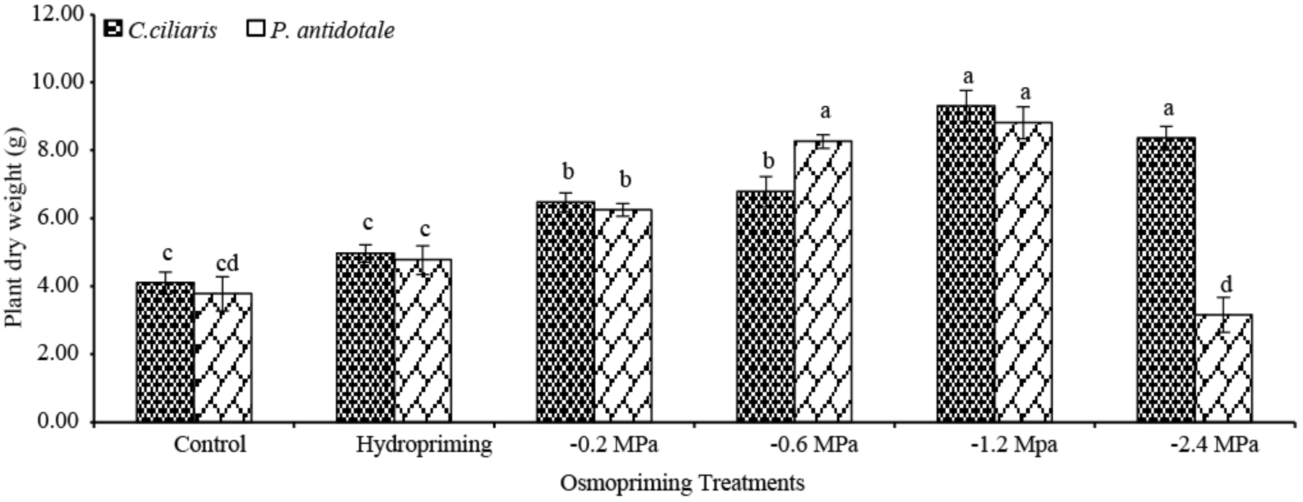

Fig. 4. Effect of PEG-8000 osmopriming on plant dry weight (g) of *C. ciliaris* and *P. antidotale*. Means showing different letters are not statistically similar ($p<0.05$) with each other. ± vertical bars represent standard errors. Data were computed from three replications consisting of three plants in each replication

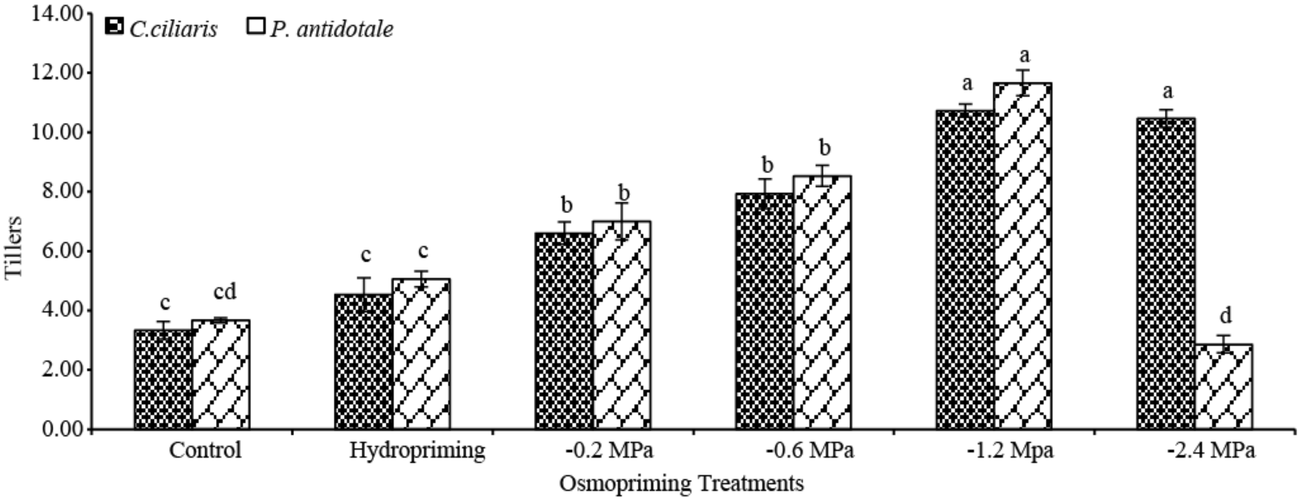

Fig. 5. Effect of PEG-8000 osmopriming on number of tillers of *C. ciliaris* and *P. antidotale*. Means showing different letters are not statistically similar ($p<0.05$) with each other. ± vertical bars represent standard errors. Data were computed from three replications consisting of three plants in each replication.

Seedling vigour: All priming treatment improved shoot length of both rangeland grasses significantly ($p<0.05$) except PEG-8000 (-2.4 MPa) treatment which reduced shoot length in case of *P. antidotale* as compared to unprimed seeds (Fig. 1). Although all treatments improved the shoot length but *C. cenchrus* seeds subjected to PEG-8000 (-2.4 MPa) priming gave the maximum value of shoot length (21.20 cm) followed by PEG-8000 (-1.2 MPa) priming (20.47 cm) as compared to control (11.99 cm). In case of *P. antidotale*, all priming treatments increased shoot length as compared to control (11.68 cm) except seeds subjected to PEG-8000 (-2.4 MPa) priming (9.71 cm). Maximum shoot length (22.73 cm) was recorded in the *P. antidotale* seeds primed with PEG-8000 (-1.2 MPa) solution followed by PEG-8000 (-0.6 MPa) priming (17.84 cm) that was statistically at par with PEG-8000 (-0.2 MPa) (17.45 cm) (Fig. 1).

Comparison of priming treatment means (Fig. 2) manifested that maximum root length (13.88 cm) was found in PEG-8000 (-2.4 MPa) primed *C. ciliaris* seeds which was statistically similar with PEG-8000 (-1.2 MPa) priming (10.77 cm) while unprimed *C. ciliaris* seeds exhibited only 4.67 cm root length. PEG-8000 (-1.2 MPa) primed *P. antidotale* plants produced maximum root length (10.16 cm) followed PEG-8000 (-0.6 and -0.2 MPa) priming (8.56 and 8.55 cm) (Fig. 2).

Figure 3.showed maximum plant fresh weight (15.81 g $plant^{-1}$) recorded in PEG-8000 (-2.4 MPa) primed *C. ciliaris* plants which was statistically similar with PEG-8000 (-1.2 MPa) priming (15.62 g $plant^{-1}$). While the minimum plant fresh weight (6.83 g $plant^{-1}$) was found in unprimed seeds while maximum dry weight of *C. ciliaris* seedling (9.30 g $plant^{-1}$) was observed in seeds primed with PEG-8000 (-1.2 MPa) priming which was statistically at par with PEG-8000 (-2.4 MPa) priming (8.36 g $plant^{-1}$) (Fig. 3). Plant fresh weight of *P. antidotale* also increased by all priming treatments except PEG-8000 (-2.4 MPa) priming which showed minimum plant fresh weight (5.48 g $plant^{-1}$) (Fig. 3). Maximum plant fresh weight of *P. antidotale* was observed in PEG-8000 (-1.2 MPa) priming (14.69 g $plant^{-1}$) followed by PEG-8000 (-0.2 MPa) priming (10.42 g $plant^{-1}$). PEG-8000 (-1.2 and -0.6 MPa) increased dry weight of *P. antidotale* plants maximally (8.81 and 8.26 g $plant^{-1}$) while PEG-8000 -2.4 MPa) primed exhibited least plant dry weight (3.16 g $plant^{-1}$) (Fig. 4). Maximum number of tillers were recorded in both range grasses when seeds were primed with PEG-8000 (-1.2 MPa) which was statistically similar with PEG-8000 (-2.4MPa) in case of *C. ciliaris* followed by -0.6 MPa OP solutions (Fig. 5).

Seed priming has been used to improve the germination/emergence, stand establishment, growth and yield of various range grasses (Hardegree & Emmerich 1992a and b; Qadir *et al.*, 2011; Nouman *et al.*, 2012). It is a pre-germination treatment in which seeds are immersed in a solution at specific water potential allowing imbibitions by avoiding radicle protrusion (Bradford, 1986). Different techniques like hydropriming, osmopriming/osmoconditioning, matripriming, hardening, osmo-hardnening, hormonal priming, etc. are in practice to prime the seeds (Hardegree, 1996; Lee *et al.*, 1998; Wu *et al.*, 1999; Farooq *et al.*, 2006a and b). It has been previously reported that range grasses' seeds are not an exception and seed priming can also improve the germination of grass species like *Festuca arundinacea* and *Dactylis glomerata* (Mauromicale & Cavallaro, 1996). PEG has been used priming different range grasses for improving germination and plant vigour (Hardegree & Emmerich, 1992a and b, 1996; Gates & Mullahey, 1997). In present study, it was found that osmopriming of *C. ciliaris* and *P. antidotale* with PEG-8000 increased emergence speed and percentage but maximum increase was observed when seeds were primed with lowest osmotic potential i.e., -2.4 MPa.

In the present study, it was found that emergence speed and percentage of range grasses was improved when seeds were subjected to PEG-8000 priming at lower osmotic potential which accelerated emergence by decreasing E50 and MET. Nouman *et al*., (2012) primed seeds of range grasses with *Moringa oleifera* leaf extract and jute mat (matripriming) demonstrated an increase in emergence potential. The researchers also observed improvement in seed vigour by PEG-8000 priming. The early and synchronized emergence might be attributed to completion of pre-germination metabolic activities which make the radical ready for extension which results into rapid and early seed emergence (Heydecker & Coolbear, 1977) or metabolic repair processes, faster production of germination metabolites, faster synthesis of RNA, proteins and osmotic adjustments, biochemical changes and enhancing physiological activities during priming treatment which are responsible for seed emergence (Bray *et al*., 1989; Sung & Chang, 1993; Basra *et al*., 2005). Hisashi & Francisco (2005) argued of decreased E50 as possible early response or *de novo* synthesis of cell wall degrading enzymes. E50 is an important emergence parameter, useful in finding out synchronization in seed emergence and plant vigour because seeds which take less time in completing 50% emergence produce more vigorous seedlings as compared to late-emerged seeds (Jahangir *et al*., 2009). In present study, significant reduction in E50 was recorded when seeds were primed with PEG solution at lower osmotic potential. Such findings were also observed by Ozbingol *et al*., (1999) who found reduction in T50 of tomato seeds when primed with PEG 8000 (-1.0 MPa) solution. No significant improvement was observed in minimizing MET of range grasses but a slight decrease was observed at low osmotic potential primed seeds. In present study, higher final emergence percentage (FEP) was recorded in *C. ciliaris* seeds when primed with PEG solution (-0.6 MPa OP) while *P. antidotale* seeds gave higher FGP when primed with -1.2 MPa OP PEG solution. These results are in accordance with Khalil *et al*., (2001) who reported that when seeds were primed with high concentrated PEG solution, those showed higher germination percentage in soybean seeds. Higher concentrated PEG-8000 has lower osmotic potential as compared to low concentrated solutions. Similarly, higher emergence index (EI) was also recorded in those seeds which were primed at lower osmotic potential. Higher emergence index (EI) is directly correlated with vigorous seedlings as reported by Jahangir *et al*., (2009). In present study, early emerged seeds resulted in vigorous seedlings as indicated by longer shoots and more fresh and dry weights. Jyotsna & Srivastava (1998) reported that osmopriming improved shoot length and seedling vigor in pigeon pea seed. Kamboh *et al*., (2000) reported that shoot growth during early seedling establishment increased when wheat seeds osmoprimed with $CaCl_2$. Vigorous seedlings may also be correlated with rapid emergence. The present study manifests that seeds which emerged early produced more biomass in comparison with late emerged seeds. Increase in shoot vigour in primed seeds might be due to enhanced and speedy seed emergence (Rehman *et al*., 2010). Ruan *et al*., (2002) also found osmopriming as helpful technique in improving radicle and plumule length in rice. They reasoned that osmopriming regulated the entry of water into seeds and also provided some nutrients which resulted in better seed performance. Moreover, Bose &Mishra (1992) and Kader & Jutzi (2002) claimed that osmopriming of wheat seeds with salt solution improved plant height, shoot and root dry weight and fresh weight. In Nouman *et al.,* (2012) findings, maximum shoot length and vigour was recorded when grasses' seeds were primed with Moringa leaf extract but PEG-8000 was also had improving effect on these seeds. Kathiresan & Gnanarethinam (1985) also reported the significant effect of osmopriming on field emergence, early and vigorous growth and some physiological processes of sunflower. They reported that osmopriming with different salts had significant effect on emergence and seedling vigour. These results also supported the findings of Ashraf & Rauf (2001) who reported that priming with $CaCl_2$ in maize seeds resulted in early germination, more final germination percentage, increased rate of germination and more? fresh and dry weight of plumule and radical. Madakadze *et al*., (1993) and Hardegree & Emmerich (1992) found the improved germination in PEG primed seeds in comparison with unprimed seeds.

In the present study, it is clear that rapid, synchronized and higher emergence and seedling vigour of *C. ciliaris* and *P. antidotale* can be obtained by priming its seeds with PEG-8000 at low osmotic potential ranging between -0.6 and -1.2.

Conclusion

From above study it can safely be concluded that the emergence rate of grass seeds, final emergence percentage of grasses, plant cover in range area, growth behavior and biomass production of rangeland grasses can be improved by subjecting their seeds with seed priming. The above technique can be helpful in development and improvement of range area. The poor economic and social condition of pastoral communities can also be improved by improving the range area.

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