Rapid and enhanced germination at low temperature of alfalfa and white clover seeds following osmotic priming

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Abstract

Seeds of alfalfa (*Medicago sativa*) and white clover (*Trifolium repens*) were primed in 3 priming solutions [2% NaCl, 2% KNO_3 and 300 g/L polyethylene glycol (PEG)] for 2 days at 20°C and their subsequent germination compared with that of untreated seed. Four replications of 50 seeds for germination and 25 seeds for emergence were arranged in a 2-factor factorial completely randomised design. All priming treatments significantly ($P<0.05$) advanced germination rate and percentage of white clover at 15°C, but produced minimal or negative effects on alfalfa. Priming with PEG significantly ($P<0.05$) reduced time to 50% of final germination percentage (G_{50} = 4.2 days) of white clover in comparison with control seeds (G_{50} = 6.5 days). The results indicated that priming seeds in KNO_3 for 2 days at 20°C could improve germination rate and germination percentage of white clover at 15°C. Possible reasons for the differing responses in the two species are discussed.

Introduction

Forage legumes are important components of sustainable agricultural systems and are important components of livestock rations. Some forage legumes have small seeds, and can be very difficult to establish in years with below-average temperatures during the germination period (Young *et al.* 1973). In addition, the low rate of elongation of juvenile seedlings of most legumes may result in poor competition with companion grasses (Kay 1968). Young *et al.* (1973) suggest that there is little possibility of selecting strains of legumes with increased capabilities for germination at low temperatures. Alternative approaches to enhancing germination rates are needed.

Alfalfa (*Medicago sativa*) is one of the most prevalent forage legumes in temperate and subtropical climates (Russelle 2001). It is normally sown in early spring when soil temperatures in temperate areas are often suboptimal for alfalfa germination and seedling growth required to establish acceptable stands, especially in marginal areas (Brar *et al.* 1991; Volenec and Nelson 1995). Germination rate and germination percentage of alfalfa are optimal when temperatures are between 15 and 25°C and seeds planted under these conditions emerge rapidly within about 17 days after planting (Brar *et al.* 1991; Volenec and Nelson 1995).

White clover is also one of the most widely grown temperate forage legumes (Frame and Newbould 1986) and is the most common legume in pastures grazed by cattle and sheep (Laidlaw and Teuber 2001). It is almost always grown in a mixture with grasses and breeding efforts aim, therefore, to optimise its contribution to the sward (Helgadottir *et al.* 2008). Years with warmer germination periods early in the season are generally referred to as 'clover years,' while late initial rains result in a cold germination period and a 'poor clover year'– conversely, a good grass year (Young *et al.* 1973). Successful establishment of juvenile seedlings of white clover following high percentage germination at low temperatures might improve competition with weedy grasses, thereby optimising its contribution to the mixture or sward (Sanderson and Elwinger 1999; Abberton and Marshall 2005; Soder *et al.* 2006).

In most tropical and subtropical climates, air temperature rarely goes below freezing, although frosts can be common, especially in elevated and inland areas (Muldoon 1986). As one moves away

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from the equator, winters become much cooler. In some places in the tropics, temperatures are far from 'tropical', with alpine tundra, tablelands (high plateaux) and the Andes at the extreme. In such areas, initial germination of several species including clovers can be reduced after imbibition at low temperatures (Hill and Luck 1991). High-yielding temperate grasses or legumes are of little use if they fail to survive cool winter temperatures, especially in the establishment year. In addition, the range of reliable legume forages available to farmers is particularly limited in those regions (Sykes 1997). Having assessed legumes and crops as suitable for the region, plant improvement studies should be initiated to obtain better levels of frost tolerance, and disease (particularly for legume crops) and insect resistance combined with satisfactory quality. Appropriate pre-sowing seed treatments might improve germination at low temperatures, in order to permit either early spring or late autumn sowings to ensure early crop establishment. This need has been pointed out for central Queensland, where only lablab (*Lablab purpureus*) and, to a limited extent, lucerne are available for use in leys (Pengelly and Conway 1998).

Seed germination of plants at low temperatures can be enhanced by specific seed treatments such as priming (Pill 1995). Priming is defined as a pre-sowing treatment with osmotic solution that allows seeds to imbibe water to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat (Heydecker and Coolbear 1977). Seed priming generally provides faster germination and field emergence with higher plant stands and earlier crop maturity, avoiding end-of-season and early-season stresses, which have practical agronomic implications, especially under adverse germination conditions such as low or high temperatures (McDonald 2000; Musa *et al*. 2001).

Priming can be accomplished through different means such as hydro-priming (soaking in water), osmo-priming (soaking in osmotic solutions such as polyethylene glycol, and sodium and potassium salts), solid matrix priming, bio-priming (coating with bacteria such as *Pseudomonas aureofaciens,* AB254) and treatment with plant growth regulators (PGRs) combined with priming medium (Chiu *et al.* 2002; Tiryaki *et al.* 2005). Development of a successful priming technique for any given plant species involves the optimising of priming temperature, water potential, duration and other conditions specific to the treatment medium (Pill 1995). Although beneficial effects of priming on seeds of forage grasses have been reported (Pill *et al.* 1997; Pill and Korengel 1997; Hardegree and van Vactor 2000; Hardegree *et al.* 2002), there is a lack of information related to seed priming of forage legumes in the literature. The objective of the present work was to determine the effects of some priming treatments on seed germination and seedling emergence of alfalfa (*Medicago sativa*) and white clover (*Trifolium repens*) at 15°C.

Materials and methods

One-year-old seeds of 'Trifecta' alfalfa (*Medicago sativa*) and 'Huia' white clover (*Trifolium repens*) provided by a seed company (Çim Teknik Tohumculuk, Ankara, Turkey) were used for experimentation. Seeds were stored at room temperature (45% RH) till used. In standard germination tests at 22°C, initial germination percentages of alfalfa and white clover were 95% and 92%, respectively, indicating that seed lots used in this experiment did not have any hard-seed dormancy.

Types of priming solution and their concentrations as well as priming duration used in this experiment were based on our previous experimental results. In those trials, priming durations of more than 2 days at given priming conditions showed intensive seed germination (radicle protrusion). Therefore, seeds were primed in darkness for 2 days at 20°C in KNO_3 (2%), NaCl (2%) or polyethylene glycol (PEG-6000, 300 g/L). Single layers of seeds were placed on double layers of filter paper saturated with 4 ml of the various priming solutions, all within covered 5.5 cm diameter petri dishes. Following priming, seeds were rinsed under running tap water for 2 minutes to remove the priming agent and air-dried under ambient room conditions on filter paper for 3 hours to remove excess water on the seed surface, and then subjected to germination tests.

Germination tests were completed in darkness in a temperature-controlled incubator held at 15 ± 0.5°C as previously reported (Young *et al*. 1973; ISTA 2004). Seeds were placed on 2 layers of filter paper moistened with 2 ml of deionised water in covered 5.5 cm petri dishes. Four replicates of 50 seeds for germination were arranged

in a 2-factor factorial completely randomised design (CRD). The treatment factors were priming medium (2% KNO_3, 2% NaCl, 300 g/L PEG and untreated control) and genotype (alfalfa and white clover). Germinated seeds (radicle visible) were recorded and removed from petri dishes daily until the numbers stabilised (complete by 10 days). From the total number of seeds germinated, final germination percentage (FGP) and its angular transformation (arcsine√FGP), days to 50% of FGP and days between 10% and 90% of FGP were calculated (Murray *et al*. 1993). Time to 50% of FGP (G_{50}) is an inverse measure of germination rate, while time between 10% and 90% of FGP ($G_{10\text{-}90}$) is considered to be an estimate of the spread of germination, the inverse of germination synchrony.

For emergence testing, 4 replications of 25 seeds from each treatment were sown into rows at 0.5 cm depth in rectangular plastic (45 x 30 x 10 cm) cups filled with peat-based growth medium. The treatments were arranged in a 2-factor factorial CRD as described above. Plastic trays were watered with tap water as needed and placed in a growth chamber at continuous temperature of 15 ± 0.5°C and under cool fluorescent lamps, which provided a photosynthetic photon flux density of 100 µmol/m^2/s for 12 h/day at seedling level. Seedling emergence (hypocotyls visible) was recorded daily until percentage emergence stabilised in all treatments (15 days). From the total number of seedlings emerged, final seedling emergence percentage (FEP) and its angular transformation, days to 10% of the FEP (E_{10}), days to 50% of the FEP (E_{50}) and days between 10% and 90% of FEP ($E_{10\text{-}90}$) were calculated. These were used to estimate emergence rate and the spread of emergence as above. After 15 days, seedlings that had emerged were cut at the surface of the germination medium and seedling fresh weight was determined.

Data from all the experiments were subjected to analysis of variance using SAS statistical software (SAS 1997), and mean separation was performed by Fisher's least significant difference (LSD) test if the F test was significant at P<0.05.

Results

Priming seeds of alfalfa and white clover for 2 days at 20°C in the presence of various osmotic solutions altered germination performance of both legumes at 15°C. All priming media significantly (P<0.05) enhanced FGP of white clover, although the same priming treatments had no effect or reduced FGP of alfalfa (Figure 1). The FGP for alfalfa seeds was significantly (P<0.05)

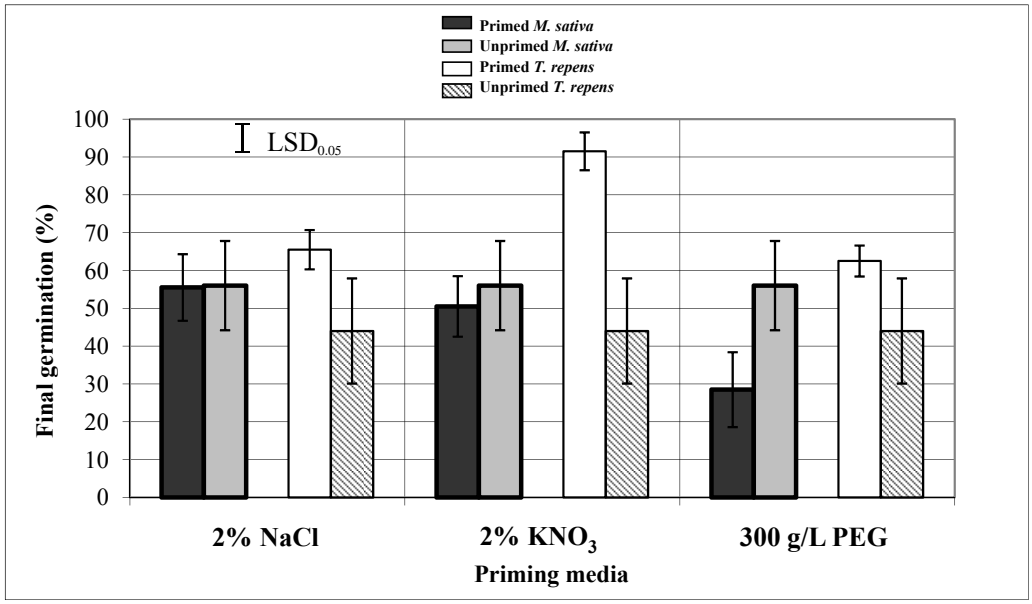

Figure 1. The effects of priming on final germination percentage of alfalfa and white clover seeds at 15°C. Seeds were primed at 20°C for 2 days in various priming media. Error bars indicate s.e. (n = 4).

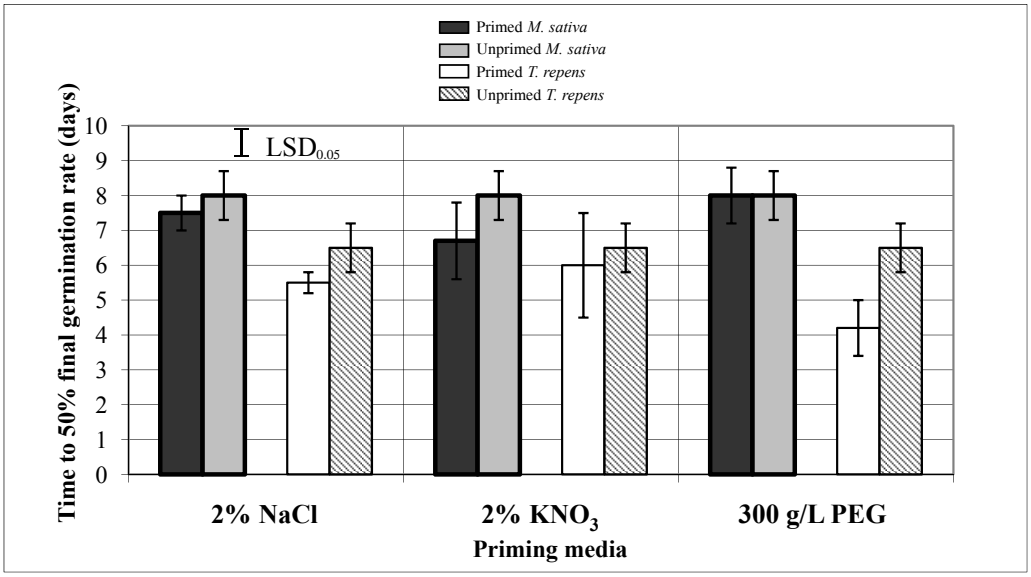

Figure 2. The effects of priming on germination rate of alfalfa and white clover seeds at 15°C. Time to 50% of final germination percentage (G_{50}) was considered to be an estimate of the inverse measure of germination rate. Seeds were primed at 20°C for 2 days in various priming media. Error bars indicate s.e. (n = 4).

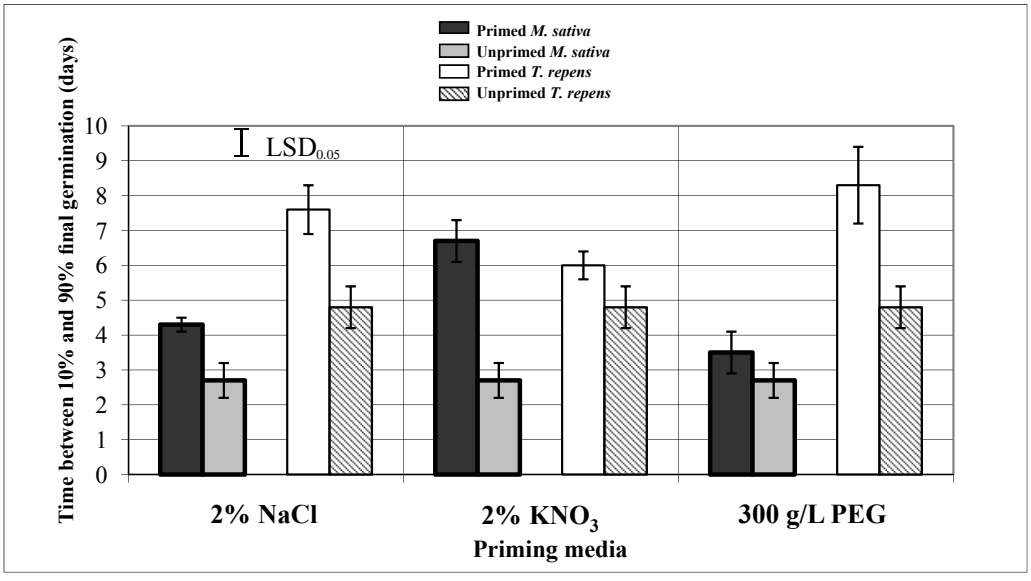

Figure 3. The effects of priming on germination spread ($G_{10\text{-}90}$) of alfalfa and white clover seeds at 15°C. Time between 10% and 90% of final germination percentage ($G_{10\text{-}90}$) was considered to be an estimate of the spread of germination, the inverse of germination synchrony. Seeds were primed at 20°C for 2 days in various priming media. Error bars indicate s.e. (n = 4).

reduced when primed in either KNO_3 or PEG with PEG causing the more severe reduction. The highest FGP was obtained from seeds of white clover primed in KNO_3 (92%), while alfalfa seeds primed in PEG had the lowest FGP (29%).

Treatment with NaCl or PEG significantly ($P<0.05$) reduced time to 50% of FGP of white clover, while treatment with NaCl and KNO_3 affected this parameter in seeds of alfalfa (Figure 2). Time between 10% and 90% of FGP (G_{10-90}) was significantly ($P<0.05$) increased by priming for both genotypes (Figure 3). Priming with KNO_3 and PEG improved low-temperature emergence of white clover compared with the control (78 and 82 *vs* 59%), but NaCl priming reduced FEP to 41.5% (Table 1). Priming with KNO_3 also significantly ($P<0.05$) reduced days to 10% of FEP compared with the untreated control. On the other hand, priming with NaCl and KNO_3 reduced FEP of alfalfa at 15°C (Table 1) from 84% in the control to 17% for NaCl and 55% for KNO_3. Priming with PEG and KNO_3, however, significantly ($P<0.05$) reduced days to 10% of FEP of alfalfa. Seedling fresh weights of alfalfa and white clover were unaffected by any of the priming treatments, while there were significant ($P<0.05$) genotype differences in seedling weight (Table 1).

Discussion

This study has provided useful preliminary data on the effects of priming seeds with a number of chemicals prior to planting on germination percentage and germination rate of alfalfa and white clover at 15°C. This adds to the bank of knowledge previously reported for other species.

It revealed that the effects of priming solutions under the test conditions can vary between plant species. While osmotic priming significantly improved FGP and germination rate as well as emergence parameters of white clover at 15°C, effects of similar treatments on germination and emergence of alfalfa seeds were minimal or negative. Increased germination rate and percentage at low temperatures following priming have also been reported for a number of other species including carrot (Pill and Finch-Savage 1988), fescue (Frett and Pill 1995), sweet corn (Chiu *et al.* 2002) and amaranth (Tiryaki *et al.* 2005). Reasons for the differences in response of the different species are not clear. Reports in the literature indicate that responses can vary with testing conditions. Kaur *et al.* (2002) reported that priming of chickpea seeds with NaCl and PEG was not effective in increasing seedling growth under conditions of water deficit. Priming of sorghum seeds with NaCl advanced germination under drought (moisture stress) but not under heat stress conditions (Kader and Jutzi 2003). It was also shown that, while salt priming resulted in faster germination rates of sorghum, it did not

Table 1. Final emergence percentage (FEP) and angular transformation of FEP [degrees], days to 10% of FEP (E_{10}), days to 50% of FEP (E_{50}), days between 10% and 90% of FEP (E_{10-90}) and seedling weight of *Medicago sativa* and *Trifolium repens* at 15°C, following priming for 2 days at 20°C in various priming media.

Genotype	Treatment	FEP		E_{10}	E_{50}	E_{10-90}	Seedling fresh weight
		(%)	[degrees]	(days)	(days)	(days)	(mg/plant)
Medicago sativa	2% NaCl	17.0	[24.1]	7.2	8.4	10.2	8.5
	2% KNO_3	55.0	[48.0]	4.2	6.5	8.0	3.4
	300 g/L PEG	84.0	[69.6]	4.8	6.8	7.7	3.2
	Untreated	84.0	[66.2]	6.4	7.0	8.3	2.4
Trifolium repens	2% NaCl	41.5	[37.5]	8.1	9.6	11.6	2.1
	2% KNO_3	78.0	[63.8]	4.4	5.8	8.3	1.8
	300 g/L PEG	82.0	[65.8]	5.0	9.1	11.1	4.6
	Untreated	59.0	[50.4]	6.0	8.5	10.1	8.2
	$LSD_{0.05}$		11.0	1.5	NS	NS	NS
	Genotype						
	M. sativa	60.0	[52.0]	5.6	7.2	8.6	12.2
	T. repens	65.1	[53.6]	5.9	8.3	10.2	5.4
	$LSD_{0.05}$		NS	NS	NS	NS	3.0

improve root or shoot growth (Kader and Jutzi 2003). Giri and Schillinger (2003) reported that none of the seed-priming media used (*i.e.*, water, KCl and polyethylene glycol) improved field emergence or subsequent grain yield in deep-planted winter wheat.

There are contradictory reports on the importance of temperature during the priming process on responses obtained. For instance, Özbingol *et al.* (1998) concluded that the optimum temperature for priming was 27 – 28°C, the same as the optimum temperature for germination of tomato seed. However, Haigh *et al.* (1986) found that temperature during priming (15, 20 or 25°C) had little effect on subsequent emergence responses of onion seeds. The different responses in the two species tested in our study when primed at 20°C for 2 days suggest that they might have different optimal temperatures in terms of response to priming. Previous reports indicated that significant advancement of germination and emergence rates as a result of priming were more pronounced at cooler temperatures (Brar *et al.* 1991; Hardegree and van Vactor 2000; Tiryaki *et al.* 2005). While germination of alfalfa can occur at 0 – 1°C (Arakeri and Schmid 1949; Tiryaki 2006), germination rate and percentage are maximised between 15 and 25°C (Brar *et al.* 1991). Priming of alfalfa seed at temperatures below 15°C might result in better responses and testing seems warranted.

Priming in KNO_3 for 2 days at 20°C resulted in increases in germination percentage of white clover at 15°C from 44 to 92%. While this priming medium and temperature might not be optimal, the responses obtained would certainly justify use of this method for enhancing germination. However, there are advantages in not achieving complete germination of seed and it is desirable to have some seed remain dormant in the soil to guard against extended dry periods following germination and failure of seedlings to establish.

Effect of salinity on seedling establishment is more conspicuous than on seed germination (Hasson and Poljakoff-Mayber 1980) and salinity inhibition of embryo-axis growth during seedling establishment might be the result of delayed mobilisation of reserves (Prisco *et al.* 1981; Gomes Filho *et al.* 1983) as well as membrane disturbance caused by salinity. Therefore, discrepancies between final germination and emergence percentages of alfalfa seeds primed in NaCl might be toxic ion effects of NaCl, rather than osmotic effects, which has been reported as the main cause of reduced germination of some other legume species (Nichols *et al.* 2009).

Polyethylene glycol is a commonly used osmotic priming material because it is readily available and has no physiological reaction with seed (Pill 1995). Very large molecules of this substance do not pass through seed cell membranes but control diffusion of water through the seed coats by providing lower osmotic potential in the seed environment (Pill 1995). Depressed FGP of alfalfa seeds following PEG priming might be a function of insufficient final water gained during priming, although similar primed seeds had a longer water imbibition period (15 days) during the seedling emergence experiment.

While many of the treatments shortened the time to 50% of final germination, this might be an artifact of the procedure followed. The priming process commenced 2 days ahead of the germination tests, so that the treated seeds were imbibing for this time ahead of the untreated seeds and would have been expected to germinate sooner even if treated with water. This suggests that a well established priming of legume seeds has the potential not only to enhance seed germination but also to provide a faster seed germination under stress conditions such as low temperature.

The most likely cause of the discrepancy between germination and emergence data of alfalfa control seeds is related to the physical constraints of the peat-based growth medium used. The degree of priming response variation owing to environmental conditions (petri or peat) suggests that the priming response of these 2 species should be further examined under a range of environmental conditions.

These findings are relevant to temperate environments where low temperatures prevail well into spring. However, in elevated situations in the subtropics and even the tropics in some cases, treatment of seeds pre-planting could allow more rapid germination of seeds and allow seedlings to establish while soil moisture levels were adequate. This could be important in reducing seedling loss when follow-up rains are delayed and could extend the growing season.

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