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Influence of temperature on germination performance of osmoprimed flue-cured tobacco (*Nicotiana tabacum* L.) seeds

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Seed enhancements such as seed priming can be used to improve germination uniformity and accelerate the rate of germination. The study focused on evaluating the interaction between light and temperature effects on germination percentages of osmoprimed tobacco seeds (*Nicotiana tabacum* L.) of eight flue-cured varieties namely K RK26, K RK28, T29, T64, T65, T66, T71 and T72. Seeds were osmoprimed for a maximum period of five days in different solutions of phytohormones (6-Benzylaminopurine (BA) and Gibberellic Acid (GA$_3$) and a salt (KNO$_3$). The lower water potential of these solutions was achieved by adding an osmoticum (Polyethylene Glycol 8000). The influence of osmotic priming was evaluated by incubating the treated seeds under light and dark conditions at optimal (20/30°C) and supra-optimal (33±2°C) temperatures. The experiment revealed that the eight tobacco varieties, gave lower germination percentages in total darkness at high temperatures (33±2°C) than when imbibed under light at 20/30°C. The non-primed seeds exhibited the least germination performance throughout the entire experiment. Osmopriming T64 and T29, in KNO$_3$+ PEG 8000 was superior in enhancing their germination percentages under dark conditions at 33±2°C. Response of the different varieties was different. K RK26, T65, T71 and K RK28 treated with BA+PEG 8000 recorded the highest germination performance for all the germination attributes used in this study whereas, T66 and T72 had a positive response to GA+KNO$_3$+PEG 8000 and BA+ KNO$_3$+PEG 8000 respectively. Osmotic priming resulted in increased germination percentages, rate of germination and improved germination uniformity of the different tobacco cultivars under supra-optimal conditions. Whereas under these conditions, the non-primed seeds of all the varieties exhibited poor germination performances. Therefore, osmotic priming can be used as a method of enhancing tobacco seed germination under stressful environmental conditions.

Key words: Osmotic priming, germination rate, germination uniformity, germination percentage, *Nicotiana tabacum* L., BA (6-Benzylaminopurine), GA$_3$ (Gibberellic acid), Potassium (KNO$_3$), Polyethylene Glycol (PEG 8000).

INTRODUCTION

Studying germination is difficult because a population of seeds does not complete the process synchronously (Still et al., 1997; Gallardo et al., 2001). In tobacco (*Nicotiana*

tabacum L.) seeds, germination is constrained by the micropylar endosperm, which covers the radicle tip, and germination can proceed if this mechanical resistance

decreases to such a level that radicle can protrude through the weakened tissues (El-Maarouf-Bouteau and Bailly, 2008). Tobacco seed does not germinate and emerge uniformly when there is poor seed to soil/media contact (Reed, 1997) and this affect uniformity in the growth of seedlings. Uniformity of growth and synchrony in development are highly desirable characters for mechanized cultural operations (Assefa, 2008) such as clipping in tobacco seedlings. Therefore, the search for improvement in the current productivity levels with reduction in production costs in tobacco culture have led to incorporation in the seedlings production system of new technologies, aiming to achieve seedlings in a shorter period of time, more vigorous and healthy, in order to improve their field performance (Almeida and Vieira, 2010). One method of improving seed germination performance both in the field and in the glass house has been through the use of presowing treatments such as priming (Assefa, 2008). Kester et al. (1997) and Abdolahi (2012) reported that this procedure can also ameliorate the detrimental effects of seed ageing. Osmopriming, refers to soaking seed in osmotic solutions such as polyethylene glycol (PEG), glycol, or mannitol (Kazemi and Eskandari, 2012). The low water potential of the treatment solution allows partial seed hydration so that pre-germination metabolic processes begin but germination is stalled (Bennett et al., 1992; McDonald, 2000; Pill and Necker, 2001). However, osmoprimed seeds may be dried back to their original moisture level (Kazemi and Eskandari, 2012) and stored for variable periods of time depending on the species (Assefa, 2008).

Priming stimulates many of the metabolic processes involved with the early phases of germination (Assefa, 2008). Gallardo et al. (2001), Harris et al. (1999) and McDonald (2000) reported that seed priming is one of the most important developments to help rapid and uniform germination and emergence of seeds, which have practical agronomic implications, notably under adverse conditions. According to Khan (1992) and Assefa (2008), osmotic conditioning in its modern sense, aims to reduce the time of seedling emergence, as well as synchronize and improve the germination percentage. Given that part of the germination processes has been initiated, the duration of the emergence period is decreased; as a result the seedlings grow faster, and more vigorously, leading to a more uniform plant stand (Assefa, 2008).

Priming helps to reduce the time course of germination (Steinmaus et al., 2000) and can also improve germination, especially when applied to poor quality seeds (Nerson, 2007). Gong Ping et al. (2000) and Finch-Savage et al. (2004) demonstrated that seed pretreatment with PEG-6000 increased seed germination and vigour index. Planting seeds treated in this way exhibit more rapid germination and reduced dormancy under adverse conditions (Cheng and Bradford, 1999).

The variable responses of different seed lots to seed priming are a continuing constraint on the commercial application of seed priming (Cheng and Bradford, 1999). Many factors, such as duration, temperature, osmotic concentrations, rehydration period, and the storage of the seed post-treatment, affect the results of the osmoconditioning treatment (Welbaum et al., 1998). The optimum condition required for osmoconditioning varies among the species as well as in relation to the osmotic condition (Assefa, 2008). The priming or osmoconditioning process, which serves to enhance seedling uniformity by improving germination and emergence, is delicate, and the stages of the process must be implemented with extreme care.

Gallardo et al. (2001) postulated that the optimization of priming treatments actually rests on carrying out subsequent germination assays, which only provide retrospective indications of the effectiveness of the priming conditions. Considerable research effort has been put into identifying how seed dormancy can best be broken to improve seed germination, but a definitive protocol is still far from having been achieved (Grundy, 2002). Primed tobacco seed is reported to germinate more uniformly, although the results are inconsistent (Hutchens, 1999; Clarke, 2001). Due to this variability in response, between seed lots, optimum priming conditions often need in practice to be determined on a case-by-case basis for many species (Halmer, 2004). Therefore this study involved laboratory components to determine seed priming effects on flue cured tobacco seed germination.

MATERIALS AND METHODS

Seed material

Pure seed lots of *N. tabacum* L., varieties T71, T72, T64, T66, T29, T65, KRK26 and KRK28 were used. These cultivars had varying degrees of dormancy ranging from very strong to moderate dormancy. The eight flue-cured varieties of tobacco seeds produced in 2009 and 2010 season were supplied by the Seed Production Division, Tobacco Research Board, and Harare, Zimbabwe.

Osmopriming solutions

Osmoticum – Polyethylene glycol 8000 (PEG 8000).

Polyethylene glycol (PEG 8000) solution was prepared according to Michel (1983) to give approximately -18.0 MPa osmotic potential at 25°C.

The priming solutions that were used are 100 mg 6-Benzylaminopurine (BA)/liter water, 100 mg Gibberellic acid (GA)/liter water, 100 mg (BA+GA), 0.2% Potassium Nitrate (KNO_3), distilled water and their various combinations thereof.

Osmopriming protocol

PEG priming of seeds was carried out by placing samples of seed (1 g) of known weight in test tubes to which 24 ml PEG 8000 (30%) mixed with salt solution or phytohormone had been added. Each test tube contained a constant amount of the priming solution (PEG,

BA, GA, KNO_3 and their various combinations thereof). The priming solution was continuously aerated by an aquarium pump. The maximum priming time in PEG 8000 mixed with different phytohormones and KNO_3 in our priming treatments was five days while those of distilled water were three days.

After the priming period, the seeds were rinsed thoroughly with distilled water to remove any PEG, surface dried on filter paper, then spread on dry blotters and left to be air dried for two days at 25°C on the lab bench. The seeds were transferred to paper packets and stored at room temperature before germination tests were conducted in water at 20/30°C or at 33±2°C.

Osmoprimed seeds were divided into two sub-samples, and one sub-sample was stored without any further treatment. The other sub-sample was subjected to heat shock (incubation at 35°C for 7 days). All the treated seeds were stored in paper envelopes at room temperature until required for sowing.

Germination assays

Response to priming was assessed by germination performance (rate, uniformity, total germination percentage, mean germination time and germination index) under dark and light conditions. Germination was expressed as the cumulative percentage of light and dark germinated seeds for both the indoors and greenhouse experiments.

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981) and Afzal et al. (2005):

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

Where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of germination.

Rate of germination (R) was calculated following modified formula:

R= 1/MGT

Uniformity (GU) was calculated following modified formula:

$$GU = \frac{\sum n}{\sum [(Fn-t)^2 n]}$$

Where t is the time in days, starting from days 0, the day of germination and n is the number of seeds germinate t and F is equal to MGT (Abdolahi et al., 2012).

Germination index (GI) was calculated as described in the Association of Official Seed Analysts (1983) as the following formulae:

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \cdots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Light and dark germination

Effects of light on germination

Seeds were incubated in 4.7 cm diameter glass Petri dishes lined with cotton wool and two layers of Whatman No. 1 filter papers wetted with 16 ml of distilled water. Germination tests were conducted under constant temperature of 33±2°C and under alternate cycles of dark (16 h)/light (8 h) and low/high temperature of 20/30°C. Germination was defined as endosperm rupture, and protrusion of the radicle. Control tobacco seeds were not primed and they were put on germination tests as described above. Daily counts were conducted for 10 continuous days.

Effects of darkness on germination

For dark germination the dishes were wrapped in two layers of aluminium foil to ensure complete darkness. The Petri dishes were placed in a controlled-temperature growth room at 33±2°C and incubator at 20/30°C for 10 days. The percentage of germinated seeds was scored under a microscope. Control tobacco seeds were not primed and they were put on germination tests as described above.

Each dish contained 100 seeds and treatments were arranged in a split plot design (within each temperature regime) with six replicates per treatment.

Data collection

Germination counts were conducted on daily basis for light imbibed seeds and for the dark imbibed seeds the germination counts were conducted on day 10. Daily temperatures were recorded during the experimental period.

Statistical analysis

Data was analyzed using Genstat statistical package, 9th edition (Lawes Agricultural Trust, Rothamsted Experimental Station). Least significant difference (LSD) at 5% for discriminating treatment means was used. Data are means of 6 replicates ± SD.

RESULTS

Effects of temperature on germination uniformity (U), germination rate (R) and total germination percentage of dark imbibed tobacco seeds was evaluated for a continuous five days growth period following priming treatments. Seeds of eight tobacco cultivars T64 (Table 1), T29 (Table 2), K RK26 (Table 3), T71 (Table 4), T65 (Table 5), K RK28 (Table 6), T66 (Table 7) and T72 (Table 8) osmoprimed for five days were incubated for five days under two light conditions (light and dark) at an alternating temperature of 20/30°C and a constant temperature of 33±2°C.

Improved performance for T64 (Table 1) and T29 (Table 2) was obtained for seeds primed with KNO_3+PEG 8000. At a temperature of 33±2°C, KNO_3+PEG treated T64 seed germinability remained consistently high with the fastest germination rate, improved GU and a germination percentage of 69% under dark conditions which was statistically different from the non-primed seed (4%) (Table 1). T29 primed with the same treatment also exhibited improved performance for all the germination attributes under the same conditions (Table 2). Dark imbibed primed T29 seeds had germination percentage of 92% which was significantly different from most of the

Table 1. Effects of osmotic priming on germination parameters of T64 tobacco seeds. Data are means six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.24971[c]	0.26487[e]	1.312[c]	0.983[b]	77.2[b]	49b[c]
GA+PEG 8000	0.2654[a]	0.28384[a]	0.98a[b]	0.744[a]	96.3[a]	52.2[bc]
KNO$_3$+PEG 8000	0.26617[a]	0.28292[a]	0.976[ab]	0.783[a]	95.7[a]	69.3[a]
BA+GA+PEG 8000	0.2566[b]	0.27387[cd]	1.148[b]	0.889[ab]	93.7[a]	50.8[bc]
BA+KNO$_3$+PEG	0.25039[c]	0.26971[de]	1.318[c]	0.933[ab]	91.3[ab]	42.8[bc]
GA+KNO$_3$+PEG	0.26645[a]	0.28106[ab]	0.954[ab]	0.796[ab]	95[a]	49[bc]
BA+GA+KNO$_3$	0.26628[a]	0.27949[ab]	0.945[a]	0.811[ab]	94.7[a]	66[ab]
Distilled H$_2$O	0.25395[bc]	0.27205[d]	1.244[c]	0.91[ab]	90.3[ab]	38.2[c]
PEG 8000	0.26392[a]	0.2773[bc]	1.004[ab]	0.842[ab]	95.2[a]	48.5[bc]
Non-primed	0.22556[d]	0.24714[f]	3.033[d]	1.502[c]	65[bc]	3.5[d]
l.s.d. treatment	0.00521		0.1983		14.56	
l.s.d. temperature	0.00233		0.0887		6.51	
F Pr.	≤0.001		≤0.001		≤0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

Table 2. Effects of osmotic priming on germination parameters of T29 tobacco seeds. Data are means six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.27194[bc]	0.28878[b]	1.111[a]	0.702[a]	85[ab]	64.5[bc]
GA+PEG 8000	0.26956[cd]	0.28169[cd]	0.917[a]	0.786[ab]	91[a]	72.7[bc]
KNO$_3$+PEG 8000	0.2794[a]	0.29771[a]	0.824[a]	0.647[a]	78.5[ab]	92[a]
BA+GA+PEG 8000	0.24431[g]	0.26593[f]	1.507[d]	1.014[b]	72[b]	54.7[c]
BA+KNO$_3$+PEG	0.26296[de]	0.27268[ef]	1.004[ab]	0.911[ab]	93.7[a]	72.8[bc]
GA+KNO$_3$+PEG	0.27717[ab]	0.28926[b]	0.834[a]	0.723[ab]	81.3[ab]	65.3[bc]
BA+GA+KNO$_3$+PEG	0.27725[ab]	0.28782[bc]	0.817[a]	0.723[ab]	96.5[a]	72.7[bc]
Distilled H$_2$O	0.25243[f]	0.271[f]	1.307[bd]	0.933[ab]	79.3[ab]	23.8[d]
PEG 8000	0.26216[e]	0.27903[de]	1.441[cd]	0.816[ab]	95.3[a]	73.7[ab]
Non-primed	0.23199[h]	0.25348[g]	2.307[e]	1.346[c]	31.7[c]	0.3[e]
l.s.d. treatment	0.00694		0.3036		18.61	
l.s.d.temperature	0.0031		0.1358		8.32	
F Pr.	<0.001		<0.001		<0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

treatments including the control which failed to germinate five days after planting (Table 2).

The highest germination performance for K RK26 (Table 3), T71 (Table 4) and T65 (Table 5) was obtained from seeds primed with BA+PEG 8000 and this was significantly different from their respective non-primed seeds. Priming K RK26 (Table 3) with BA+PEG 8000 resulted in improved GU and increased R under both temperature conditions. The final germination percentage of the dark imbibed seeds was high at 93±4% averaged over all treatments except for the seeds primed with distilled water and the non-primed seeds which had germination percentages of 77 and 56% respectively (Table 3) at a constant temperature of 33±2°C. The same trend was also observed for T65 seeds primed with the same solution (Table 4). The final germination percentage (96±2%) of the dark imbibed T65 seeds was significantly high at 20/30°C for all treatments compared to seeds primed in distilled water (77%) and the non-primed seeds (64%) (Table 4). Incubation at low

Table 3. Effects of osmotic priming on germination parameters of K RK26 tobacco seeds. Data are means six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.29648[a]	0.30989[a]	0.64[a]	0.571[a]	93.5[a]	89.5[a]
GA+PEG 8000	0.28954[abc]	0.30891[abcd]	0.725[a]	0.594[a]	97[a]	95.33[a]
KNO_3+PEG 8000	0.28596[bcd]	0.30304[bcd]	0.759[a]	0.635[a]	96.33[a]	95.17[a]
BA+GA+PEG 8000	0.29301[ab]	0.30861[abcd]	0.684[a]	0.595a	96.17[a]	95.83[a]
BA+KNO_3+PEG	0.27647[e]	0.3056[abcd]	1.087[b]	0.613[a]	96.5[a]	94.67[a]
GA+KNO_3+PEG	0.29054[ab]	0.30933[abc]	0.712[a]	0.588[a]	96.5[a]	95.5[a]
BA+GA+KNO_3+PEG	0.28294[cde]	0.3023[cd]	0.781[a]	0.642[a]	92.67[a]	95.17[a]
Distilled H_2O	0.28934[abc]	0.31064[a]	0.727[a]	0.581[a]	94.83[a]	76.83[b]
PEG 8000	0.28149[de]	0.30165[d]	0.788[a]	0.636[a]	97.17[a]	94.5[a]
Non-primed	0.24599[f]	0.26709[e]	1.553[c]	1.065[b]	94.17[a]	55.67[c]
l.s.d. treatment	0.00748		0.1668		7.882[NS]	
l.s.d. temperature	0.00334		0.0746		3.525	
F Pr.	<0.001		<0.001		<0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

Table 4. Effects of osmotic priming on germination parameters of T65 tobacco seeds. Data are means of six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.29917[a]	0.29791[a]	0.6118[a]	0.6335[a]	94.5[a]	92.7[a]
GA+PEG 8000	0.28948[bc]	0.29045[bcd]	0.6891[b]	0.6841[b]	95[a]	80.3[b]
KNO_3+PEG 8000	0.28923[bcd]	0.29143[bcd]	0.6915[b]	0.6963[b]	96.3[a]	95.2[a]
BA+GA+PEG 8000	0.29906[a]	0.29508[ab]	0.607[a]	0.6495[ab]	97.5[a]	89.8[ab]
BA+KNO_3+PEG	0.29104[b]	0.29251[bc]	0.6732[b]	0.6818[b]	96.7[a]	93.5[a]
GA+KNO_3+PEG	0.28469[de]	0.2892[cd]	0.7517[cd]	0.7008[c]	97.3[a]	86.5[ab]
BA+GA+KNO_3+PEG	0.28507[cde]	0.29087[bd]	0.7051[b]	0.6958[b]	94.5[a]	86.3[ab]
Distilled H_2O	0.28434[e]	0.28774[d]	0.7088[bc]	0.6956[b]	77.2[b]	23[d]
PEG 8000	0.27814[f]	0.27825[e]	0.7669[d]	0.7783[d]	94.2[a]	64.7[c]
Non-primed	0.2407[g]	0.24409[f]	1.6669[e]	1.5684[e]	63.8[c]	17.5[d]
l.s.d. treatment	0.00471		0.04826		12.38	
l.s.d. temperature	0.0021		0.02158		5.53	
F Pr. treatment	<0.001		<0.001		<0.001	
F Pr. temperature	0.120		0.421		<0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

temperatures resulted in a reduced germination percentages of the T65 seeds. Under these conditions BA+PEG 8000 treated seeds had a germination percentage of 93% which was significantly different from the seeds primed with distilled water, PEG 8000 and the non-primed seeds which had germination percentages of 23, 65% and 18% respectively (Table 4).

Priming T71 seeds in distilled water resulted in increased germination speed and improved germination uniformity compared to all other treatments (Table 5).

Although priming with distilled water improved germination under light conditions, the germination percentages (83% at 20/30°C and 40% at 33±2°C) of this seed lot was significantly lowered under dark conditions for both temperature levels. BA+PEG 8000 treated seeds were consistently high for the same germination parameters and this treatment resulted in high germination percentages (95% at 20/30°C and 73% at 33±2°C) for the dark imbibed seeds which were significantly different from the seeds primed with distilled

Table 5. Effects of osmotic priming on germination parameters of T71 tobacco seeds. Data are means of six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.283^b	0.28374^b	0.695^b	0.697^b	94.5^a	72.67^a
GA+PEG 8000	0.25747^{de}	0.26318^d	1.079^{ef}	0.99^d	95.67^a	55.17^{bc}
KNO$_3$+PEG	0.25468^e	0.25767^e	1.169^f	1.127^e	95.83^a	56.83^{bc}
BA+GA+PEG	0.27599^c	0.27715^c	0.765^{bc}	0.747^{bc}	97^a	59.83^b
BA+KNO$_3$+PEG	0.27206^c	0.2748^c	0.783^{bcd}	0.766^{bc}	96.67^a	49.33^c
GA+KNO$_3$+PEG	0.26204^d	0.26442^d	0.99^e	0.945^d	95.67^a	49^c
BA+GA+KNO$_3$+PEG	0.27155^c	0.2731^c	0.828^{cd}	0.821^c	96.5^a	70.17^a
Distilled H$_2$O	0.2909^a	0.29424^a	0.601^a	0.597^a	82.67^b	39.5^d
PEG 8000	0.24713^f	0.25544^e	1.316^g	1.13^e	92.17^a	26^e
Non-primed	0.23892^g	0.23949^f	1.704^h	1.751^f	93.67^a	23.33^e
l.s.d. treatment	0.0048		0.0908		8.532^{NS}	
l.s.d. temperature	0.00215		0.0406		3.816	
F Pr. treatment	<0.001		<0.001		<0.001	
F Pr. temperature	0.008		0.084		<0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

Table 6. Effects of osmotic priming on germination parameters of K RK 28 tobacco seeds. Data are means of six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.30013^{ab}	0.30307^a	0.589^a	0.595^a	94.3^a	86.8^{abc}
GA+PEG 8000	0.29563^c	0.29718^b	0.638^{ab}	0.626^a	93.2^{ab}	81.3^{bc}
KNO$_3$+PEG 8000	0.29085^d	0.29273^c	0.696^{abc}	0.678^{ab}	96.5^a	93.3^a
BA+GA+PEG 8000	0.30401^a	0.30449^a	0.586^a	0.593^a	98.5^a	79.8^c
BA+KNO$_3$+PEG	0.29579^c	0.30331^a	0.626^{ab}	0.599^a	98^a	94.7^a
GA+KNO$_3$+PEG	0.28994^d	0.29761^b	0.691^{ab}	0.639^{ab}	98.3^a	87.3^{abc}
BA+GA+KNO$_3$+PEG	0.29651^{bc}	0.30074^{ab}	0.638^{ab}	0.609^a	96.3^a	89.5^{ab}
Distilled H$_2$O	0.26709^f	0.2704^e	0.936^c	0.869^b	84.3^b	14.2^e
PEG 8000	0.27341^e	0.27629^d	0.861^b	0.83^{ab}	96.8^a	62.7^d
Non-primed	0.22075^g	0.22714^f	3.497^d	3.021^c	26.8^c	0.2^f
l.s.d. treatment	0.00425		0.2425		9.23	
l.s.d. temperature	0.0019		0.1084		4.13	
F Pr. treatment	<0.001		<0.001		<0.001	
F Pr. temperature	<0.001		0.203		<0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

water and the non-primed seeds (23% at 33±2°C) (Table 5).

Mixing BA and GA in PEG 8000 promoted germination speed and uniformity of primed K RK28 seeds, but this trait did not significantly differ from BA+PEG treated seeds (Table 6). Just like K RK26 (Table 3), T71 (Table 4) and T65 (Table 5), K RK28 seeds treated with BA+PEG resulted in improved GU and increased R under both temperature conditions (Table 6). Priming K RK28

seeds with BA+GA and in BA only all in combination with PEG resulted in increased germination percentages under dark conditions at high temperature (33±2°C) which were significantly different from the seeds primed with distilled water (14%), PEG (63%) and the non-primed seeds (0%) (Table 6). There was, however, no statistical difference ($P \leq 0.05$) between seeds primed with BA+GA and the BA treated seeds (Table 6).

Although priming T66 tobacco seeds with distilled

Table 7. Effects of osmotic priming on germination parameters of T66 tobacco seeds. Data are means six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.25728[d]	0.27332[f]	1.133[c]	0.878[c]	93.33[b]	78.17[c]
GA+PEG 8000	0.28369[bc]	0.30125[bc]	0.769[b]	0.631[ab]	96.5[ab]	88.83[b]
KNO$_3$ +PEG	0.2848[bc]	0.30327[bc]	0.769[b]	0.62[ab]	95.67[ab]	95.33[a]
BA+GA+PEG	0.28719[bc]	0.30634[b]	0.734[b]	0.596[ab]	97.17[ab]	96.83[a]
BA+KNO$_3$+PEG	0.28749[bc]	0.29998[cd]	0.74[b]	0.63[ab]	95.5[ab]	94.83[a]
GA+KNO$_3$+PEG	0.28932[b]	0.30622[b]	0.725[b]	0.598[ab]	98.83[a]	95.67[a]
BA+GA+KNO$_3$+PEG	0.27692[d]	0.29505[de]	0.833[b]	0.666[b]	97.33[ab]	97.5[a]
Distilled H$_2$O	0.32471[a]	0.32426[a]	0.518[a]	0.519[a]	96.83[ab]	85.83[b]
PEG 8000	0.28295[c]	0.29179[e]	0.764[b]	0.701[b]	97.83[ab]	85.17[b]
Non-primed	0.23957[e]	0.26319[g]	1.899[d]	1.121[d]	88[c]	10.17[d]
l.s.d. treatment	0.00592		0.131		5.09	
l.s.d. temperature	0.00265		0.0586		2.276	
F Pr.	<0.001		<0.001		<0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

Table 8. Effects of osmotic priming on germination parameters of T72 tobacco seeds. Data are means six replicates of the light (R and GU) and dark germinated seeds under two temperature regimes.

Treatment (s)	R		GU		Dark germination %	
	20/30°C	33±2°C	20/30°C	33±2°C	20/30°C	33±2°C
BA+PEG 8000	0.28562[d]	0.2901[de]	0.7272[cd]	0.6834[bc]	93.7[abc]	85.7[a]
GA+PEG 8000	0.27513[e]	0.27889[f]	0.8195[e]	0.7777[d]	97.7[a]	58[c]
KNO$_3$ +PEG 8000	0.27904[e]	0.28575[e]	0.7929[de]	0.7309[cd]	97[ab]	67.8[bc]
BA+GA+PEG 8000	0.29279[bc]	0.29887[bc]	0.6454[abc]	0.6089[ab]	97.3[ab]	81.7[a]
BA+KNO$_3$+PEG	0.30647[a]	0.31281[a]	0.5785[a]	0.5573[a]	97.2[ab]	91.2[a]
GA+KNO$_3$+PEG	0.28734[cd]	0.28783[e]	0.703[bc]	0.708[cd]	96.8[ab]	71[b]
BA+GA+ KNO$_3$+PEG	0.29558[b]	0.30131[b]	0.632[ab]	0.608[ab]	97[ab]	86.8[a]
Distilled H$_2$O	0.29128[bcd]	0.29534[cd]	0.6426[ab]	0.6164[ab]	86.5[c]	38.3[d]
PEG 8000	0.26592[f]	0.26869[g]	0.9583[f]	0.9377[e]	94.7[abc]	45.3[d]
Non-primed	0.24133[g]	0.25021[h]	1.6577[g]	1.3959[f]	87.5[bc]	10.5[e]
l.s.d. treatment	0.00573		0.08216		9.92	
l.s.d. temperature	0.00256		0.03674		4.43	
F Pr. treatment	<0.001		<0.001		<0.001	
F Pr. temperature	<0.001		0.005		<0.001	

R = Germination rate; GU = Germination uniformity. Data with the same letters are not significantly different ($P \leq 0.05$).

water resulted in improved R and GU the dark imbibed seeds of the same treatment exhibited reduced germination percentage (86%) at 33±2°C (Table 7). This was significantly lower than that of the seeds primed with GA+KNO$_3$ (96%). Under the same conditions non-primed seeds had a total germination percentage of 10% which was significantly lower than all other treatments (Table 7).

Priming T72 seeds with BA+KNO$_3$+PEG resulted in improved GU (0.5785 at 20/30°C and 0.5773 at 33±2°C), increased germination speed (0.306479 at 20/30°C and 0.31281 at 33±2°C) and percentage (92%) under dark conditions at high temperature (33±2°C) which were significantly different from the seeds primed with distilled water (14%), PEG (63%) and the non-primed seeds (0%) (Table 6).

DISCUSSION

Germination of osmoconditioned seeds was determined to assess its relationship with germination rate, uniformity and germination percentage under both light and dark

conditions at two temperature levels (Tables 1 to 8). Primed seeds of eight tobacco cultivars (T71, T72, T64, T66, T29, T65, KRK26 and KRK28) and their controls were incubated for five days at an alternating temperature of 20/30°C and a constant temperature of 33±2°C. A definite trend toward increased germination performance by combining/mixing the phytohormones and or the inorganic salt KNO_3, with PEG 8000 was evident compared to PEG 8000 alone. Bakht et al. (2011), Bonvissuto and Busso (2007) and Farashah et al. (2011), in their studies found out that PEG 6000 and 8000 only, improved germination performance of various crop species. Unlike from the findings of this study it is evident that the influence of PEG 8000 on germination can be further improved by mixing with phytohormones. To further confirm this, in some studies by Khan (1992) and Assefa (2008) they observed that osmoconditioning had an incremental effect on the germination rate, uniformity of emergence, and the capacity of seeds to withstand adverse environmental conditions. Ghassemi-Golezanik et al. (2008) reported that osmopriming contributes to significant improvement in seed germination and seedling growth in different plant species. The response of the different cultivars in this study differed with respect to the increase in germination rate, uniformity and the total germination percentage under dark conditions (Tables 1 to 8). Similar results were obtained by Lanteri et al. (1994) who observed that the effect of a given osmotic treatment differed between seed lots of the same pepper cultivar. Hartley et al. (2001a) also reported different responses to priming in the flue-cured cultivars NC 71 and NC 72.

High temperature (33±2°C) had a promotory effect on the germination, performance of light imbibed seeds with germination proceeding more rapidly for the primed seeds compared to the non-primed seeds (Tables 1 to 8). The germination speed at both temperatures (20/30 and 33± 2°C) following priming was dependent on the priming treatment. However, seed germination rate was significantly affected by seed priming for all the varieties. For all treatments, germination proceeded most rapidly in the full-light treatment except for the non-primed which showed slow germination speed under both temperatures (Tables 1 to 8). It is surprising that germination was slowest in the incubator (20/30°C), where we had the optimum temperatures for germination, but it should be kept in mind that this condition comparably performed better than the 33±2°C condition in darkness (Tables 1 to 8). At a temperature of 20/30°C under dark conditions seed germinability was consistently high at around 91% averaged over all species (Tables 1 to 8). This further confirms Hartley et al. (2002) suggestion that, the light requirement for the germination of some tobacco cultivars and other photodormant species can be bypassed by alternating the day/night temperature during germination to mimic a typical diurnal fluctuation. Thus, it remains to be determined whether the main factor responsible for

the reduced germination under high temperature in dark was thermoinhibition or the small range of temperature fluctuation. The fact that non-primed seed actually performed well in dark under 20/30°C than in darkness under high temperature implies that inhibition was involved. Considerable increases in germination rates were evident in primed seeds. Seeds primed in BA + osmotica (PEG 8000) and then incubated at 33±2°C exhibited fast germination speed at this supra-optimal temperature (Tables 3, 4, 5). This confirms Hutchens (1999) and Clarkes' (2001) findings, that primed tobacco seed germinate faster and maintain higher germination rates at a wider temperature range than non-primed seed. KNO_3 was effective in improving all the germination parameters of T64 (Table 1) and T29 (Table 2), similar to results from previous studies in tomato seeds by Kester et al. (1997).

Basra et al. (2007) reported that primed seeds usually exhibit increased germination rate, greater germination uniformity, and sometimes greater total germination percentage. This was further strengthened by the findings of this study where priming significantly improved germination uniformity of all the varieties compared to the non-primed seeds (Tables 1 to 8). Hartley et al. (2001a, b) demonstrated that tobacco plants that emerged two days apart were significantly different in size, and therefore homogeneity at 55 days of age. Hartley et al. (2001a) and Clarke (2001) also reported that the survivability of later germinating plants is lowered. Germination tests document seed viability, but not non-uniform emergence, which influences seedling vigor (Clarke, 2001). Uniformity is weighted more heavily than size because a uniform small population can be managed more effectively than a large non-uniform population (Clarke, 2001) (Table 3a, b, c, d, e).

The germination percentages of non-primed seeds were lower as compared to seeds from primed seeds (Tables 1 to 8). Similar germination difficulties were observed in seeds treated with distilled water and PEG 8000 (Tables 1 to 8). The effect of seed priming on germination percentage of tobacco seeds was significant. While some priming treatments like BA+KNO_3+PEG 8000 (Table 5) and BA+KNO_3+PEG 8000 and KNO_3+PEG 8000 (Table 6) resulted in large germination percentages they had no significant effect on germination rate. Exclusion of light at a temperature of 33±2°C reduced the germination percentages of both the primed seeds and the non-primed seeds, but the total germination of the primed seeds exceeded that of the controls (non-primed and PEG 8000 treated seeds) (Tables 1 to 8). This indicates that seed priming can alleviate the negative effects of supraoptimal temperatures. Orzeszko-Rywka and Podlaski (2003) found out that priming alone and or in combination with rubbing improved seed tolerance to unfavourable environmental conditions. Rapid seed germination and stand establishment are critical factors for crop production under stress conditions (Rouh et al., 2011).

Although, there was germination for all the treatments and there were significant differences in total germination percentages of most of the treatments. Germination at 20/30°C under dark conditions was statistically similar for almost all the treatments for each respective variety (Tables 1 to 8). Non-primed seed at 20/30°C had a mean germination percentage of 69 averaged over all varieties and this was significantly different to that at 33±2°C which was below 17%. Priming with distilled water alone had significant effect on R and GU (Tables 3, 5, 7) but had minimal effect on germination percentage throughout the entire osmotic priming experiment. Limited information on BA as germination enhancing treatment suggests that its potential has not been fully explored in seed priming.

Conclusion

Seed priming in this experiment appears to be a useful technique to break dormancy and improve seed germinability even under suboptimal conditions. Increasing knowledge of the molecular mechanisms of dormancy will reveal further ways to improve seed technologies and seed quality. Priming in this experiment resulted in a significant increase in germination performance. Therefore the future looks bright for innovative research and practice in this field, and the opportunity for success appears to be great. An important advantage is the availability of research and measurement instrumentation as a basis for immediate progress for those entering the program.

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