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EFFECT OF TEMPERATURE REGIMES, DORMANCY BREAK TREATMENTS AND LOW HYDRATION ON SEED GERMINATION IN BARLEY, TOMATO AND TURNIP

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SUMMARY

Germination of surface sterilized seeds of barely, tomato and turnip was tested under different temperature regimes (constant temperatures : 16, 21 and 31°C) and alternate temperatures (10/30°C (16h/8h), 20/30°C (16h/8h), 15/25°C (16h/8h)), in filter paper lined paired petridishes with 12 h/d light inside BOD incubator. Effect of dormancy break treatments on promotion of seed germination and effect of low hydration (–1.5 MPa) maintained by sucrose, mannitol or polyethylene glycol (MW 6000) solutions and abscisic acid (25 mg/l) on seed germination was examined under various temperature regimes in darkness, in 16 h/d or in continuous light provided by fluorescent tubes (400 fc). Each type of seed tested was found to have a specific germination temperature range and most effective dormancy break treatment varied. For barley, it was 15°C, dehusking; in tomato, germination in alternate temperature 20/30°C (16h/8h) in light and for turnip 21°C in light and GA_3 200 to 400 mg l^{-1} (co-applied).

Key words: **Barley, dormancy, hydration, seed germination, temperature, tomato, turnip.**

INTRODUCTION

During the period from seed sowing to seedling establishment, seed is exposed to a wide range of environmental factors that can adversely affect germination performance which could be slow or erratic. Time of maturity and marketable yield in many crops is influenced by the time taken for seeds to germinate and seedlings to establish. In seeds that dehydrate at maturity (orthodox) and are non-dormant, germination is initiated by imbibition of water. Water uptake during seed germination is generally triphasic and sensitive to factors that influence timing and extent of germination of a particular seed. ψ_b is threshold or base water potential of a seed that will just prevent germination at given percentage. In soil, seed is exposed to natural fluctuations in soil moisture. Radicle emergence is an irreversible commitment for individual seed to germinate but there is extended quiescence at subcritical water potential. Radicle emerges only if water content of seed after imbibition exceeds a critical value ψ_b. Hilhorst (1993) has suggested that as a function of time and temperature, a precursor of Pfr receptor is synthesized and is available only if temperature is suitable for germination. Seed dormancy (primary or secondary) interferes with results of germination tests. In some species, successful prescriptions for germination tests have been developed. However, there are still many species where techniques for dormancy removal are unsatisfactory. Moreover, the seed lot could be either of non-dormant, high quality genetically homogenous cultivar or genetically heterogenous cultivar or of poor quality. This study investigates most suitable temperature regime and successful dormancy break prescriptions as well as the effect of low hydration and exogenous abscisic acid on germination of three morphologically distinct seed types.

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* L), tomato (*Lycopersicon esculentum* Mill cv. Solan Gola) and turnip (*Brassica rapa* L cv. Purple Top) were procured from local market. Since origin of seed lots was not known, gemination was tested under constant temperature of 16, 21 and 31°C, in 12 h/d light in filter paper lined paired petridishes inside BOD incubator. Non-germinated seeds at the end of germination test were calssified as viable or non-viable, on the basis of tetrazolium chloride test, mouldy and empty. Suitable germination temperature for each seed type was recorded. Each set of experiment was repeated twice. Association of Official Seed Analysts (AOSA) and International Seed Testing Association (ISTA) recommendations for germination test period is 14 to 56 days as both aged and dormant seeds may begin to germinate after a substantive delay. However, if progress of germination is very slow, it might be better to terminate the germination test after 2-3 weeks and pursue next step in the algorithm.

Barley is endospermic caryopsis enclosed in floret. AOSA and ISTA have recommended 7-15°C as germination temperature and reomoval of seed covering either by acid scarification or dehusking as successful dormancy break treatments. Tomato seed coat is thin, has a light requirement for oxygen permeability and it possesses an endodermal cap opposite radicle tip which must be weakend and penetrated on germination by radicle. AOSA and ISTA recommended light, 0.2% KNO_3 (co-applied) treatment as successful dormancy break treatments for tomato seed germination. Alternate temperature 20/30°C (16h/8h) in 16 h/d or in continuous light or 20 to 30°C constant temperature are recommended as suitable.

Turnip seed is non-endospermic with mucilaginous seed coat that restricts oxygen availability on imbibition. Testa is split during imbibition. Constant temperature from 20 to 30°C or alternate temperatures of 10/30°C (16h/8h); 20/30°C (16h/8h) or 15/25°C (16h/8h) in 16 h/d light is recommended for promotion of turnip seed germination. GA_3, 200-400 mg/l (co-applied) treatment is also reported to have a positive effect.

Effect of low hydration maintained by sucrose, mannitiol or PEG (MW 6000) and abscisic acid (25 mg/l) on germination of each seed type was recorded. In barley, starch concentration was estimated by iodine stain assay (Preiss 1982) in above mentioned treatments after four days. Standard starch solution concentration was 5 mg/ml and colour intensity was measured at 660 nm on a digital spectrophotometer. Concentration of starch in unknown solution was calculated in comparison with standard. Each set of experiment was repeated twice.

RESULTS AND DISCUSSION

For barley, 16°C and for tomato and turnip 21°C in 12 h/d light was found to be most suitable germination

Table 1. Cumulative germination percentage and classification of non-germinated seeds after germination test duration (28 days) in constant temperature regimes and 12 h/d light (100 seeds per test, n = 10)

Temperature regime		Cumulative germination percentage ± S.E. (at end of test)	Non-germinated seeds at the end of test (expressed as percentage of original number of seeds tested)			
			Viable	Non-viable	Mouldy	Empty
16°C	Barley	40±3.5	52	2	-	6
	Tomato	-	90	2	-	8
	Turnip	8±2.6	53	43	2	2
21°C	Barley	20±3.2	58	10	4	8
	Tomato	60±5.5	20	8	2	10
	Turnip	34±4.8	18	30	2	16
31°C	Barley	2±1.3	44	10	40	4
	Tomato	18±3.8	46	10	12	20
	Turnip	6±1.2	36	30	18	10

- represents no germination or nil.

temperature (Table 1). However, presence of large percentage of viable seeds (barley : 52, 58, 44; tomato : 90, 20, 46 and turnip : 53, 18, 36% at 16, 21 and 31°C constant temperature regimes, respectively) suggested that seed dormancy was interfering with the results of germination tests. Moreover, turnip seed lot was of poor quality (non-viable seed percentage : 43, 30 and 30% at 16, 21 and 31°C, respectivley) on the basis of tetrazolium chloride test. At 31°C, barley seed germination was very poor (Table 1) and a large proportion of seeds placed for germination became mouldy. Walker-Simmons (1990) had reported enhancement of abscisic acid sensitivity in wheat embryo at higher temperature (30°C as compared to 15°C).

Since, for barley, a constant germination temperature range of 7.5 to 15°C and removal of seed coverings is recommended (ISTA, 1976), barley seed was either scarified in concentrated sulfuric acid for 2.5, 4 and 5 minutes or dehusked manually. For promotion of germination, dehusking was more effective than acid scarification (Table 2), especially at temperature 15°C.

Table 2. Effect of concentrated H_2SO_4 scarification and dehusking on cumulative germination % of barley seeds under different temperature regimes in darkness after 10 days (100 seeds per test, n = 10)

Temperature (°C)	Cumulative germination (% ± S.E.) Scarified for			Dehusked (% ± S.E.)
	2.5 min	4 min	5 min	
7.5	5±1.35	18±1.9	28±1.4	55±4.1
10	2±1.2	28±2.8	46±3.3	70±3.8
15	10±2.4	40±1.42	72±2.6	95±2.7

0.2% KNO_3 (co-applied), 2% thiourea (pre-applied for 16h), 20 mg/l Kinetin (pre-applied for 16h) and 100 mg/l GA_3 (co-applied) were tested as dormancy break treatment for husked, scarified (5 min. in conc. H_2SO_4) or dehusked barley seeds (Table 3). Thiourea treatment suppressed germination of scarified seed maximally and cumulative germination per cent at the end of the test was lower than in water even in husked seeds (Table 3) probably due to its toxic effect. Germination per cent was in general lower

Table 3. Effect of 0.2% KNO3 (co-applied), GA_3 100 mg/l (co-applied), Kinetin 20 mg/l (pre-applied) for 16 h and thiourea 2% (pre-applied for 16h) on cumulative germination % of barley seeds after 10 days at 15°C in darkness (100 seeds per test, n = 10)

Treatment	Cumulative germination (% ± SE)		
	Husked seeds	Scarified (5 min. in conc. H_2SO_4)	Dehusked seeds
0.2% KNO3	15±2.4	68±2.6	78±3.3
2% Thiourea	20±1.9	2±0.9	38±3.6
GA_3 100mg/l	38±3.9	76±3.8	94±1.9
Kinetin 20mg/l	43±1.7	70±2.8	92±1.7
H_2O	38.±2.2	74±1.9	88±3.6

with KNO_3 treatment and higher with GA and kinetin treatments than in water (88%).

Thus in barley, removal of seeds coverings and suitable temperature are most effective in promoting germination. However, low hydration [maintained by 0.6 M polyethylene glycol (MW 6000), sucrose (0.6 M) or mannitol (0.6 M)] and (25 mg/l) co-applied abscisic acid suppressed starch mobilization during germination of dehusked barley seed at 15°C as compared to seed germination in water (Table 4). Robertson *et al.* (1989) have reported accumulation of an endogenous ∝ amylase inhibitor that accumulates during barley grain development and is specific for GA inducible

Table 4. Effect of low hydration (-1.4 Mpa) co-applied and abscisic acid (25 mg/l) co-applied, on starch mobilization during germination of dehusked barley seeds at 15°C constant germination temperature in darkness after 4 days.

Treatment	Concentration of starch (mg/g imbibed seed) (on basis of starch iodine stain assay)
PEG 0.6 M (MW 6000)	591.2±4.7
Sucrose 0.6 M	577.1±5.2
Mannitol 0.6 M	683.5±6.4
ABA 25 mg/l	531.0±6.1
H_2O	127.7±8.1

∝ amylase isozyme in aleurone tissue. Its synthesis can be increased by ABA or water stress or nutrition or temperature during germination. ABA and high osmoticum were reported to prevent germination of embryos or in cultures (Kermode 1990) but high osmotica may not inevitably include an elevation in ABA content of embryos or in plants. Roberts *et al.* (1993) reported that a subset of LEA (Late Embryo Genesis abundant proteins, involved in desiccation tolerance) proteins is induced in all seedling tissues in barley subject to water stress. Mobilization of storage reserves within seed is an germinative event and in cereals, it is initiated by a GA inducible ∝ amylase enzyme whose formation is inhibited by ABA at transcription level (Jacobsen and Close 1991). Indentical effects were reported by high osmotica but there was no accompanying elevation of endogenous ABA (Garcia Maya *et al.* 1990). Regulation of LEA gene transcription by ABA or osmotica (–1.4 to –1.6 Mpa) is reported to be complex (Hilhorst *et al.*, 1993). In some species, both have synergistic effect, in others have separate transducing pathways. In barley, it was observed that both ABA and high osmotica, prevented starch mobilization during germination. Hetherington and Quatrano (1991) had suggested that LEA gene regulation is via protein kinases that phosphorylate or dephosphorylate transcription factors with possible involvement of calcium ions.

Tomato seed coat is thin and has light requirement for oxygen permeability. Constant germination temperature of 15 to 30°C or alternate temperature of 20/30°C (16h/8h) in light (200-400 fc) has been recommended (ISTA 1976, AOSA 1981) for promotion of tomato seed germination. It was observed that alternate temperature of 20/30°C (16h/8h) in continuous or 16h/d light are effective in promoting tomato seed germination (Table 5) as compared to tomato seed germination at 20°C constant temperature in 16h/d light (Table 1). However, light seems essential for tomato seed germination as indicated by poor cumulative germination per cent (20% in 20/30°C; 16h/8h) in dark. GA_3, 200 and 400 mg/l promoted tomato seed germination in dark but germination was not promoted by 0.2% KNO_3 in dark (Table 5). Low hydration (-1.4 Mpa to -1.6 Mpa maintained by 0.6 M sucrose, 0.6M mannitol and 0.6 M PEG coapplied) and 25 mg/l ABA co-applied suppressed tomato seed germination at 20/30°C (16h/8h) in 16h/d light (Table 6). Seeds in ABA or mannitol solutions were not mouldy while majority of those in sucrose or PEG solution were mouldy at the end of germination test. Hilhorst (1993) had speculated that during conditions conducive to germination, the precursor of Pfr receptor is synthesized, its cofactor is NO_3^- and seed germination is restricted to those periods when receptor is active or available. GA receptor sensitivity was also affected by light and temperature but not in reversible manner. GA synthesis and sensitivity are part of Pfr triggered transduction pathway leading to germination which is absolutely dependent on exogenous or endogenous GA (Berry and Bewley 1992). Xu *et al.* (1990) had reported that high osmotica (-1.5 to -1.6 Mpa) can substitute for ABA in suppressing precocious germination of embryo in developing seeds. However, there is no evidence that ABA is resynthesized when seeds enter secondary dormancy (Derkx 1993). In tomato, mobilization of reserve food starts before visible protrusion of radicle. GA_{4+7} was reported to

Table 5. Effect of alternate temperature regimes in dark or in light, 0.2% KNO_3 (co-applied) and GA (co-applied) on cumulative germination (%) of tomato seeds after 12 days (100 seeds per test, n = 10).

Germination test regime	Cumulative germination (% ± SE) at end of the test			
	0.2% KNO_3	GA 200 mg/l	GA 400 mg/l	H_2O
20/30°C (16h/8h) in darkness	23±1.9	70±1.4	58±4.4	20±3.8
20/30°C (16h/8h) in 16h/d light	75±2.8	65±2.4	70±4.1	55±1.2
20/30°C (16h/8h) in continuous light	59±3.3	60±3.8	65±4.7	60±2.3

Table 6. Effect of low hydration and abscisic acid in 20/30°C (16h/8h), 16 h/d light on cumulative germination % of tomato seeds after 12 days (100 seeds per test, n = 10).

Treatment	Cumulative germination % (at end of the test)
H_2O	58±1.9
Mannitol 0.6 M	-
Sucrose 0.6 M	-*
PEG 0.6 M (MW 6000)	-*
ABA 25 mg/l	1±0.3

-Represents no germination

*Seeds mouldy at end of the test

increase activity of cell wall hydrolysing enzyme which were inhibited by ABA (Kermode 1990). It is not known yet whether, GA and ABA share a common but antagonistic site of action on embryo growth during germination.

GA is reported to promote turnip seed germination. Light, KNO_3 in combination, also promote germination at 15 to 30°C temperature range (AOSA 1981). However, GA (co-applied) promoted (18-34%) germination of turnip seed and 0.2% (co-applied) KNO_3 suppressed (2-4%) it when compared to seeds placed for germination in water (8-18%) at alternate temperature regimes of 10/20°C, 20/30°C and 15/25°C for 16h/8h (Table 7). However, germination at 21°C constant temperature was 34% in water (Table 1). Low hydration maintained by co-applied 0.6M PEG (MW 6000), 0.6 M sucrose and 0.6 M mannitol and 25 mg/l (co-applied) abscisic acid, suppressed germination compared to seeds placed in water for germination at 21°C in continuous light (Table 8).

Table 7. Effect of alternate temperature regimes and 0.2% KNO_3 (co-applied) and GA (co-applied) on cumulative germination % of turnip seed under continuous light after 12 days (100 seeds per test, n = 10).

Temperature regime	Cumulative germination, % ± SE (at end of the test)			
	0.2% KNO_3	GA 200 mg/l	GA 400 mg/l	H_2O
10/30°C (16h/8h)	2±0.9	23±2.1	18±1.9	8±1.7
20/30°C (16h/8h)	4±1.4	32±2.2	34±2.4	16±2.1
15/25°C (16h/8h)	2±0.5	31±3.1	33±2.1	18±2.8

Table 8. Effect of low hydration (-1.4 MPa) co-applied and 25 mg/l co-applied abscisic acid at 21°C constant germination temperature, in light, on cumulative germination % of turnip seeds after 12 days (100 seeds per test, n = 10).

Treatment	Cumulative germination (% ± S.E.)
PEG 0.6 M (MW 6000)	-
Sucrose 0.6 M	-
Mannitol 0.6 M	-
ABA 25 mg/l	1±0.5
H_2O	35±1.5

-Represents no germination

Thus, each type of seed tested had a specific germination temperature range and the most effective dormancy break treatment varied. For barley, it was 15°C, dehusking; in tomato, germination in alternate temperature 20/30°C (16h/8h) in light, and for turnip, 21°C in light and GA_3 200 to 400 mg/l (co-applied). Plant development, in general, is regulated by changes in sensitivity of cells and tissues to environmental and hormonal signals. Amount, by which regulatory factor exceeds its sensitivity to threshold value, determines the rate at which regulated process proceeds (Bradford *et al.* 1993). Come and Corbineau (1992) had reported that transition into and out of secondary dormancy may continue for years before seeds germinate or decay. However, low hydration and abscisic acid suppressed germination of each seed type under optimum germination temperature regimes. Skriver *et al.* (1991) have suggested that both may act on inhibition of extension growth by restricting water uptake. ABA may also be inhibiting germinative metabolism as reported by Berry and Bewley (1992).

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