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Assessment of soybean *(Glycine max)* vigour by the seed leachate conductivity assay

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Abstract

Conductivity of seed leachates is inversely correlated with germination and growth of soybean, *Glycine max* (L.) Merr., at low temperatures. It is concluded that the seed leachate conductivity assay can be employed to assess soybean seed vigour. Cold tolerance of soybean can markedly be enhanced by increasing the hydration level of the seeds to at least 20% by means of exposure of the seeds to HRH (water saturated atmosphere) for 4 days. It is suggested that increased vigour following HRH exposure is due to re-establishment of the cell membrane integrity in the course of exposure.

INTRODUCTION

Germination of seeds on synthetic substrates under optimum conditions is poorly correlated with seedling emergence in the field, especially in early spring sowings (Perry, 1972; Woyke and Ostrzycka, 1975). A much better indicator of the planting seed quality is what is called seed or seedling "vigour" (Franck, 1950; Isely, 1957). According to Heydecker's definition, "Vigour is the cover name given to that complex of interrelated properties which render seeds versatile enough to germinate and flourish under a wide range of conditions" (Heydecker, 1977). Of many tests recommended for determining the seed vigour (Woodstock, 1973), the simplest and the most reliable seems to be the seed leachate conductivity assay (Hibbard and Miller, 1928) which has been successfully used to indicate seed stocks of poor planting value in peas (Matthews and Bradnock, 1968; Bedford, 1974; Woyke and Ostrzycka, 1975) and field beans (Hegarty, 1977). The test may also be applied to diagnose the drought resistance of *Brassica napus* L. (Długokęcka and Kacperska-Palacz, 1978). Electrolyte leakage was reported to be a good indicator of vigour of isolated embryo axes in soybean (Yaklich and Abdul-Baki, 1975).

It has been found that intact soybean seeds differing as regards germinability at low temperatures also markedly differ in respect to conductivity of seed leachates (Knypl and Janas, 1979). The aim of this study was to assess the conductivity test as a potential method of evaluating the vigour of soybean.

MATERIAL AND METHODS

Seeds

Low-vigour seeds of soybean, *Glycine max* (L.) Merr. cv. 'Warszawska', and high-vigour seeds of cv. 'Traverse' were the test objects. The seeds (harvest 1976) were stored at 7—9°C. At time of the experiments (May-September, 1977) the seeds contained *ca.* 9.8% of water per dry weight basis.

Infusion of pesticides to dry seeds

To prevent growth of microflora the seeds were infused with acetone solution of 1% thiram (tetramethylthiuram disulfide) and 1 mM chloramphenicol (CAP) for 4 h, blotted on a filter paper and dried under reduced pressure for 1 h (Khan et al., 1973).

HRH and LRH exposure

Batches of 120 seeds in punched paper envelopes were hung in glass exsiccators containing either distilled water and folded filter paper on the bottom to increase the evaporation area (HRH exposure) or dry blue silica gel (LRH exposure). After 4 days at 25°C the seeds were weighed and immediately used for conductivity and germination assays. Dry weight was determined after the seeds had been kept for 48 h at 105°C.

Conductivity assays

Thirty seeds were placed in 150 ml of triple distilled water which had previously been cooled to 10°C or warmed to 25°C, and kept at these temperatures, respectively. Conductivity of the steep water was measured with OK-102/1 (Radelkis, Budapest) conductometer. The temperature of solutes during conductivity assays was maintained the same as during seed soaking.

Germination and growth

Fourty seeds were placed in 11-cm Petri dishes on two discs of Whatman No. 1 filter paper which had been wetted with 20 ml of distilled water, and germinated at constant temperature (12 or 14°C) and illumination (4.6 W m^{-2}). Radicle protrusion was a germination criterion. Seedlings without visible malformations were regarded as viable.

In emergence experiments, 10 seeds were sown into 12-cm plastic pots filled with a mixture of peat moss (STK-2) and sand (1 : 3, w/w). Seeds were covered with 2.5—3 cm layer of this mixture and placed in a phytochamber KTLK 1250 (Ilka, Nema) at 14°C and 16 h photoperiod (28 W m^{-2}) at 80% RH. Illumination was produced by HQRG-400 Hg-lamps and electric bulbs (VEB Narva Berliner Glühlampen Werk, Berlin).

In field experiments, the seeds were sown in soil on April 26, 1977. Seedling emergence was counted for following 40 days.

Each laboratory experiment was repeated 3—4 times in triplicate. Results were evaluated statistically either according to Student's *t*-test or Duncan's multiple range test.

RESULTS

Conductivity of seed leachates varies in soybean cultivars, and depends on both temperature and time of imbibition (Fig. 1). The rate of electrolyte leakage was highest during the initial hour of imbibition, and then declined (*cf.* Simon and Raja Harun, 1972). Leachate conductivity was around 30—60% higher when the seeds were imbibed at 25°C as compared with that at 10°C, the difference being higher for soybean cv. 'Warszawska' than for cv. 'Traverse'. Seeds of the latter cultivar leached about three times less electrolytes within a given time and temperature of imbibition, as compared with electrolyte leakage from the seeds of cv. 'Warszawska'. When conductivity after 24 h was taken as 100%, the seeds of soybean cv. 'Traverse' and cv. 'Warszawska' as compared with the high-vigour seeds of cv. 'Traverse' the initial 2 hours of soaking. The period of high rate of electrolyte release was, thus, much longer in the case of low-vigour seeds of cv. 'Warszawska' as compared with the high-vigour seeds of cv. "Traverse" (Fig. 1).

Conductivity of any electrolyte solution depends on temperature during the assay. An increment of 2% per 1°C is characteristic for majority of salts (Podhorecka and Podhorecki, 1978). The same was true for soybean seed leachates. Seeds had been soaked for 20 h at 25°C, the steep water decanted and cooled to 5°C. Conductivity was measured at intervals during subsequent stepwise warming of the solution. Conductivity at 7.5 and 24°C equalled 300 and 400 μS cm^{-1}, respectively. This means a rise of 6 μS per 1°C or 2% increment per centigrade (Fig. 2).

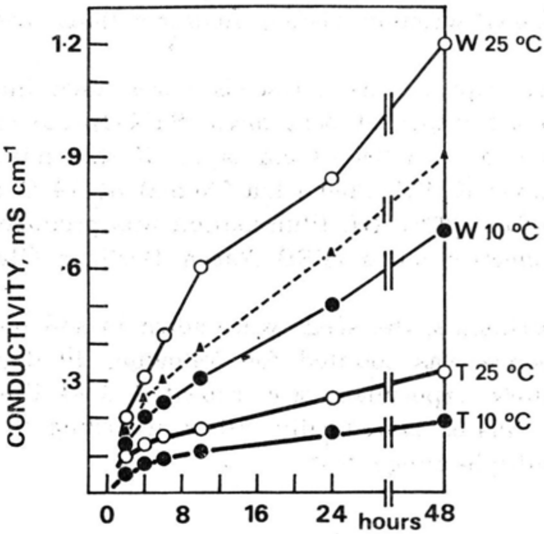

Fig. 1. Effect of temperature during imbibition on conductivity of soybean seed leachates. 30 seeds of cv. 'Warszawska' (W) or cv. 'Traverse' (T) in 150 ml water were incubated either at 10 or 25°C, and conductivity was assayed at the same temperatures. ———, seeds of cv. 'Warszawska' were soaked at 10°C and conductivity measured at 25°C

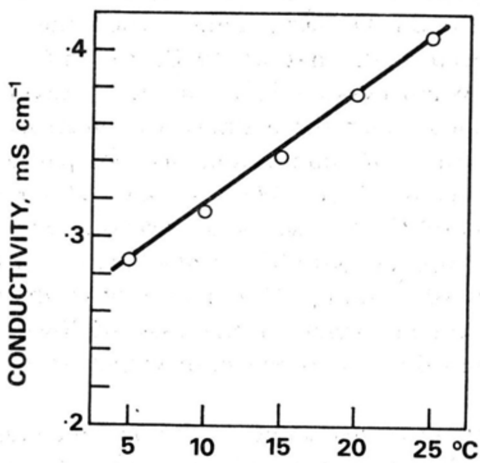

Fig. 2. Effect of temperature on conductivity. Seeds of soybean cv. 'Warszawska' (1977 harvest) were soaked for 20 h at 25°C, then the steep water was decanted and cooled to 5°C. Conductivity was measured in the course of stepwise warming of the solution

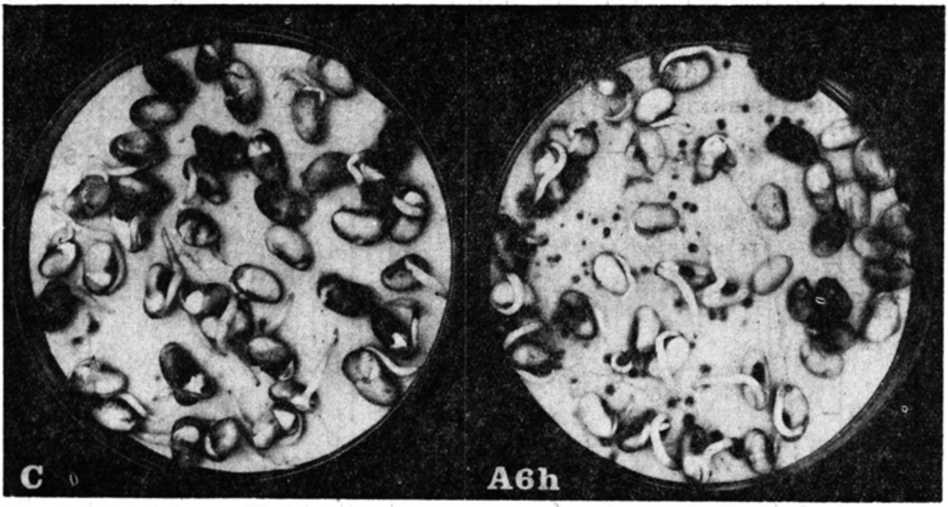

Fig. 3. Stimulatory effect of infusion of pure acetone for 6 h (A6h) on growth of microflora on low-vigour seeds of soybean cv. 'Warszawska', germinated for 9 days at 10°C. C, non-infused control seeds (Photo: W. Maliński)

Seeds of soybean cv. 'Warszawska' were heavily contaminated with fungi and bacteria after prolonged germination at low temperatures (Fig. 3). CAP added to water in which the seeds were soaked had no effect on conductivity of seed leachates during the initial 24 h, and decreased it after 48 h (Table 1). Similar results were produced by

Table 1

Conductivity of soybean seed leachate as affected by chloramphenicol (CAP) and thiram (T) Seeds soaked in water (1, 3, 4) or in 1 mM solution of CAP at 25°C. Seed batches Nos. 3 and 4 were infused with pure acetone (No. 3) or with acetone solution of T (1%) and CAP (1 mM; No. 4). Differences between any two data followed by different letters within each soybean cultivar are significant at the 1% probability level

Treatment of seeds	Conductivity ($\mu S\ cm^{-1}$) after 24 or 48 h			
	cv. 'Warszawska'		cv. 'Traverse'	
	24 h	48 h	24 h	48 h
1. Control	810 a	1200 c	250 a	330 b
2. CAP	780 a	980 b	240 a	300 b
3. Infusion with acetone	940 b	1240 c	310 b	390 c
4. Infusion with acetone solution of T and CAP	800 a	1040 b	240 a	290 b

infusion of CAP and thiram to dry seeds. It should be stressed, however, that the treatment with acetone alone enhanced both conductivity of seed leachates and growth of microorganisms upon subsequent germination at 10°C (Fig. 3). Infusion of CAP and thiram markedly prevented the growth of microflora. It seems obvious that microorganisms may enhance conductivity of seed leachates in the course of prolonged soaking.

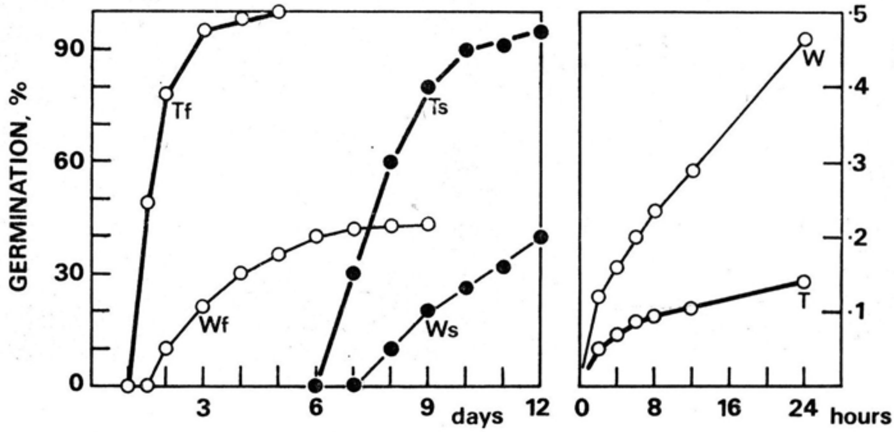

Fig. 4. Kinetics of germination of soybean seeds cv. 'Warszawska' (W) and cv. 'Traverse' (T) on filter paper (f) in Petri dishes, and emergence from peat moss--sand mixture (s). Experiments were carried out at 12°C (f) and 14°C (s). Conductivity of seed leachates (right) was measued at 10°C

Table 2

Viability and growth of syobean seedlings as measured after 8 days at 12°C. Kinetics of germination is presented in Fig. 4. The per cent of viable seedlings is expressed in relation to 100% of original seeds placed in Petri dishes. The percentage of stands in the field was counted on the 40th day after sowing the seeds in soil

Cultivar	% viable seedlings	% germination in Petri dishes	Length, mm		% stands in the field
			Hypocotyl	Root	
'Warszawska'	36	43	15	10	34
'Traverse'	98	100	27	39	87

The germination percentage on filter paper and emergence from peat moss-sand mixture at low temperatures was 95—100% and 40—45% for the seeds of cv. 'Traverse' and cv. 'Warszawska', respectively (Fig. 4). Conductivity of leachates was 140 and 465 μS cm^{-1}, respectively, when the seeds of soybean cv. 'Traverse' and cv. 'Warszawska' had been soaked for 24 h in water at 10°C. Germinability at low temperatures is obviously inversely correlated with leakage of electrolytes during imbibition in cold water. Almost all 'Traverse' seedlings were viable and they grew twice as vigorously as the seedlings of soybean cv. 'Warszawska' (Table 2). In the field only 34% of seedlings cv. 'Warszawska' emerged and remained viable in comparison with 87% in the case of cv. 'Traverse'.

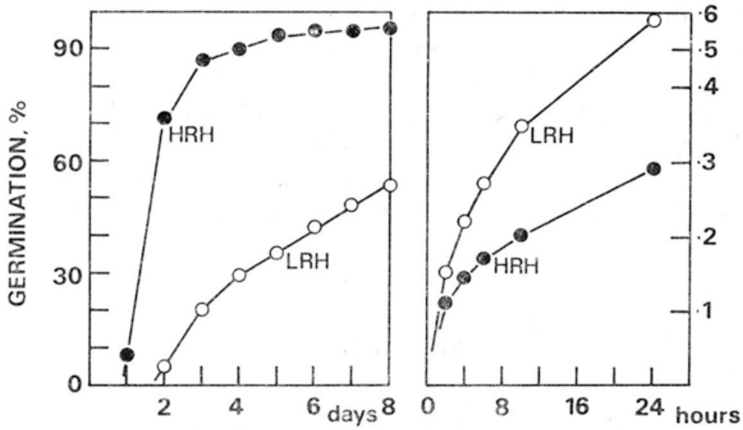

Fig. 5. Effect of HRH and LRH exposure (4 days at 25°C) on germination (14°C) of soybean cv. 'Warszawska', and conductivity of seed leachates. Seeds were infused with 1% thiram and 1 mM CAP before exposure to HRH or LRH; conductivity assays performed at 10°C; growth of seedlings presented in Table 3

Table 3

Increased soybean seed vigour following HRH exposure.

Seeds of soybean cv. 'Warszawska' were infused with acetone solution of 1% T and 1 mM CAP for 4 h, then exposed for 4 days in water-saturated atmosphere (HRH) or kept under silica gel (LRH) at 25°C. Seeds were germinated at 14°C and seedling length was measured after 8 days. Kinetics of germination is presented in Fig. 5. Moisture content in original air-dry seeds: 9.8% per dry wt basis. The per cent of viable seedlings is expressed in relation to 100% of original seeds placed in Petri dishes

Exposure	Wt. of seeds after 4-day exposure, % of initial air-dry wt.	% viable seedlings	Length, mm	
			Hypocotyl	Root
LRH	98	10	11	9
HRH	109	75	34	22

Seed vigour of many species is profoundly dependent on the initial seed hydration level Hegarty, 1978). Too dry seeds were subjected to water stress and chilling injuries upon imbibition in cold water (Pollock, 1969; Pollock et al., 1969; Perry and Harrison, 1970; Rowland and Gusta, 1977). Seed vigour can markedly be enhanced by increasing the hydration level of the seeds to a certain minimum, rather specific for a given plant species. The minimum hydration level for intact seeds of soybean which prevents the appearance of chilling injury symptoms, was determined to be 13—14% (Hobbs and Obendorf, 1972) or 20—22% (Knypl and Janas, 1979). When seeds of cv. 'Warszawska' were exposed for 4 days in water-saturated atmosphere they absorbed 9% of H_2O per original air-dry weight, i.e. at the end of HRH exposure the seeds contained *ca.* 20% of moisture per dry weight basis. HRH exposure markedly decreased leakage from the seeds and increased the germination percentage at low temperatures (Fig. 5). The percentage of viable seedlings rose to 75% and growth of hypocotyls and roots was accelerated 2.5- to 3-fold (Table 3). Exposure in dry atmosphere (LRH) markedly reduced both the percentage of viable seedlings and the seedling growth.

DISCUSSION

Soybean is a warm climate plant (Czerwiński, 1951; Lakhanov and Balachkova, 1978). Nevertheless, this plant can be adapted to cool climates (Holmberg, 1973) including the climate of Poland (Szyrmer and Federowska, 1975). Breeders need a rapid and simple test to assess cold tolerance of seed material.

Since the quantity of electrolyte released by seeds upon soaking in water inversely correlates with both germination and growth at low temperatures and field performance, the assay can be used to assess the degree of resistance of seeds of new lines and mutants of soybean to low temperatures. Results of the conductivity test are highly reproducible, if temperture and time of imbibition are constant, and if temperature during the conductivity assay is controlled. Triple-distilled water should be used and the time of soaking should not be too long. Otherwise growth of microorganisms should be prevented by adding antibiotics and/or fungicides to the soaking water. In that case the reference sample, i.e. the sample without seeds, should contain the same concentration of antibiotics. The aim of the reference sample is to correct the results of conductivity assays for minor fluctuations caused by solubilization of CO_2 in water during the imbibition period.

The test may be used for determining the vigour of individual seeds. If soaking lasted not more than 24 h, the seeds after the leaching assay could be used for germination (data not shown).

Why does HRH exposure increase the soybean seed vigour? Lipids of cell membranes in dry seeds persist in "leaky" hexagonal state (Simon, 1974). In such state the membranes are not semipermeable and they seem disorganized upon inspection in an electron microscope (Webster and Leopold, 1977). At a certain hydration level — specific for the plant species, the membrane phospholipids adopt a lamellar configuration which is characteristic for semi-permeable membranes (Simon, 1974; Bramlage et al., 1978). Such a structural rearrangement of membrane phospholipids possibly takes place during

HRH exposure, as inferred from the electrolyte leakage reduced to one half of the control rate. Pollock (1969) found that HRH exposure of lima bean seeds (*Phaseolus lunatus* L.) nullified the symptoms of chilling injury upon subsequent germination in cold water as compared with 100% mortality among the seeds exposed over $CaCl_2$. Similar results were noted in experiments with seeds of *Allium porrhum* L. and *Beta vulgaris* L. (Gulliver and Heydecker, 1973).

The regaining of membrane integrity in the course of exposure in HRH seems to be a direct cause of enhanced low-temperature tolerance of soybean seeds. The HRH-exposed seeds leak also less amino acids and sugars, and become less contaminated with moulds and bacteria upon germination at low temperatures (data not shown). This may be one of the secondary factors responsible for increased emergence of soybean seedlings and increased yield in field conditions (Knypl, 1980) because it is well established that seed exudates may contain substances stimulatory for growth of microflora (Schroth and Cook, 1964).

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Przewodnictwo wód nastoinowych miarą wigoru nasion soi (Glycine max)

Streszczenie

Zdolność nasion soi odm. 'Warszawska' i odm. 'Traverse' do kiełkowania i wschodów oraz wzrostu w niskich temperaturach jest odwrotnie skorelowana z przewodnictwem wód nastoinowych. Wnioskuje się, iż test przewodnictwa może być stosowany do prognozowania wschodów soi w warunkach niskich temperatur i wysokiej wilgotności gleby.

Wigor soi można podwyższyć przez wstępną ekspozycję nasion w atmosferze nasyconej parą wodną (HRH). Wzrost zawartości wody w nasionach do 20% pociąga za sobą spadek przewodnictwa wód nastoinowych o 50%; kiełkowanie w niskich temperaturach ulega przyspieszeniu, podnosi się liczba siewek żywotnych oraz 2—3-krotnie zwiększa się wzrost hypokotyli i korzeni. Przypuszcza się, iż przyczyną wzrostu wigoru nasion spowodowanego ekspozycją HRH jest przywrócenie integralności strukturalnej a tym samym i cechy selektywnej przepuszczalności membranom cytoplazmatycznym. Strukturalna przebudowa składnika fosfolipidowego membran cytoplazmatycznych ze stanu heksagonalnego do stanu lamellarnego pociąga za sobą spadek wrażliwości na stres wodny z jednoczesnym zwiększeniem odporności na porażenie przez mikroflorę.