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Polyethylene Glycol 6000 Priming Effect on Germination of Aged Wheat Seed Lots

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Abstract. The effects of PEG 6000 priming on germination performance of aged wheat seed lots have been studied. A correct application of osmopriming treatment indicated a relationship between the pattern of water absorption, the reactivation of mitotic activity and the start and synchronization of germination. The possibility of controlling pregerminative events by means of this treatment is discussed on the physiological basis of seed germination.

Hydration and subsequent dehydration treatment on seeds has been described as a method of improving germination performance and viability during storage (HANSON 1973, SAVINO *et al.* 1979). Controversal results on germination of fresh harvested and old seed lots can be due to rapid water uptake. Soaking injury may be overcome by presowing equilibration with a humid atmosphere or by osmotic "priming" treatment up to germination by means of aqueous solutions of osmotica (the more used is PEG 6000, a high molecular mass inhert polymer) (HEYDECKER and COOLBEAR 1977). By a series of combinations of chemical concentration (resulting in osmotic potential), temperature and duration of treatment, a progressive arrest of visible germination and synchronization of the following germination, when seeds are replaced in distilled water, may be induced (AKALEHIYWOT and BEWLEY 1977). A prolonged pregerminative time is normal in aged seed lots which need a longer period than fully viable seeds to start germination and to reach the maximum germination percentage. A correct application of osmopriming treatment may enable aged seeds to retain and then restore their germinability more effectively, as suggested by HEYDECKER *et al.* (1975). In this way limitation of water absorption could affect the recovering of metabolic functionality by a more efficient reactivation of repair mechanisms leading to membrane and macromolecular integrity (BERJAK and VILLIERS 1972).

In this paper the effects of PEG 6000 priming on germination performance of wheat seed lots at different final germination percentages are reported. The possible physiological basis of advantageous effects of osmopriming

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treatments on aged seed germination are discussed in the light of the potential utility of this method in a more extensive study of the components of germination process.

MATERIAL AND METHODS

Stocks of wheat seeds (*Triticum durum* cv. Appulo, 1981 harvest) at various final percentages of germination were obtained by storage at 13.5 to 14% of moisture at 30 °C, according to the method of ROBERTS and ABDALLA (1968). In these conditions four different seed lots were prepared within six weeks: 92% G (1981 harvest), 86% G, 73% G and 62% G, respectively.

The osmotic potentials of PEG 6000 (purchased from Merck) solutions were calculated according to MICHEL and KAUFMANN formulas (1973).

Osmopriming treatments were performed at times and temperatures as indicated in the result section. 100 seeds were incubated in 12 cm Petri dishes containing two sheets of Whatman No. 1 filter paper imbibed with 25 ml of one of the PEG solutions. After the priming period the seeds were collected, rinsed several times with distilled water and dried with filter paper. The seeds were then immediately spread in 9 cm Petri dishes (twenty seeds each) containing two sheets of Whatman No. 1 filter paper moistened with 10 ml of distilled water for further imbibition at 20 °C in the dark. A seed was considered as germinated when the coleoptile and a normal primary root or, in its absence, at least two lateral roots were well visible.

Water uptake was measured by the basic method of an air-oven on dissected embryo (or seedling) and on the remaining grain.

Samples of five embryos (or seedlings), isolated from seeds subjected to PEG priming treatment and to successive imbibition in distilled water, were fixed in Carnoy and stained by the Feulgen method. The primary root was cut off and a squash preparation was made on a slide; five-six hundred cells per root were observed for studying mitotic reactivation. The mitotic index was expressed as percent value of the ratio: observed methaphases/total number of cells scored (DELTOUR and JACQUARD 1974).

RESULTS

Preliminary experiments established the most appropriate conditions of PEG priming to obtain germination advantage in a relatively short time. Moreover, short duration of treatment and low concentration of PEG avoided fungi contamination, excessive viscosity of osmotic solutions and the occurrence of an acceptable degree of pregermination (COOLBEAR *et al.* 1980). Osmotic potentials of −0.38 MPa and −1 MPa (0 MPa is referred to pretreatment in distilled water) were applied on the seed lots for the duration of 2 days at 20 °C and 4 days at 5 °C. Fig. 1 provides an overall picture of germination curves of seed lots with decreasing viability subjected to osmopriming treatment. There was no significative gain in the final percentage of germination in all seed lots considered. The evident result of combination of 2 days at 20 °C treatment was the increase of germination rate together with the anticipated start of germination within the first day of imbibition in

Abbreviations used: PEG 6000-polyethylene glycol 6000; %G-germination percentage.

distilled water compared with germination of unprimed seeds. In these experiments the germination curves were well differentiated with osmotic potentials ranging from −0.38 MPa to −1 MPa within the first day of water

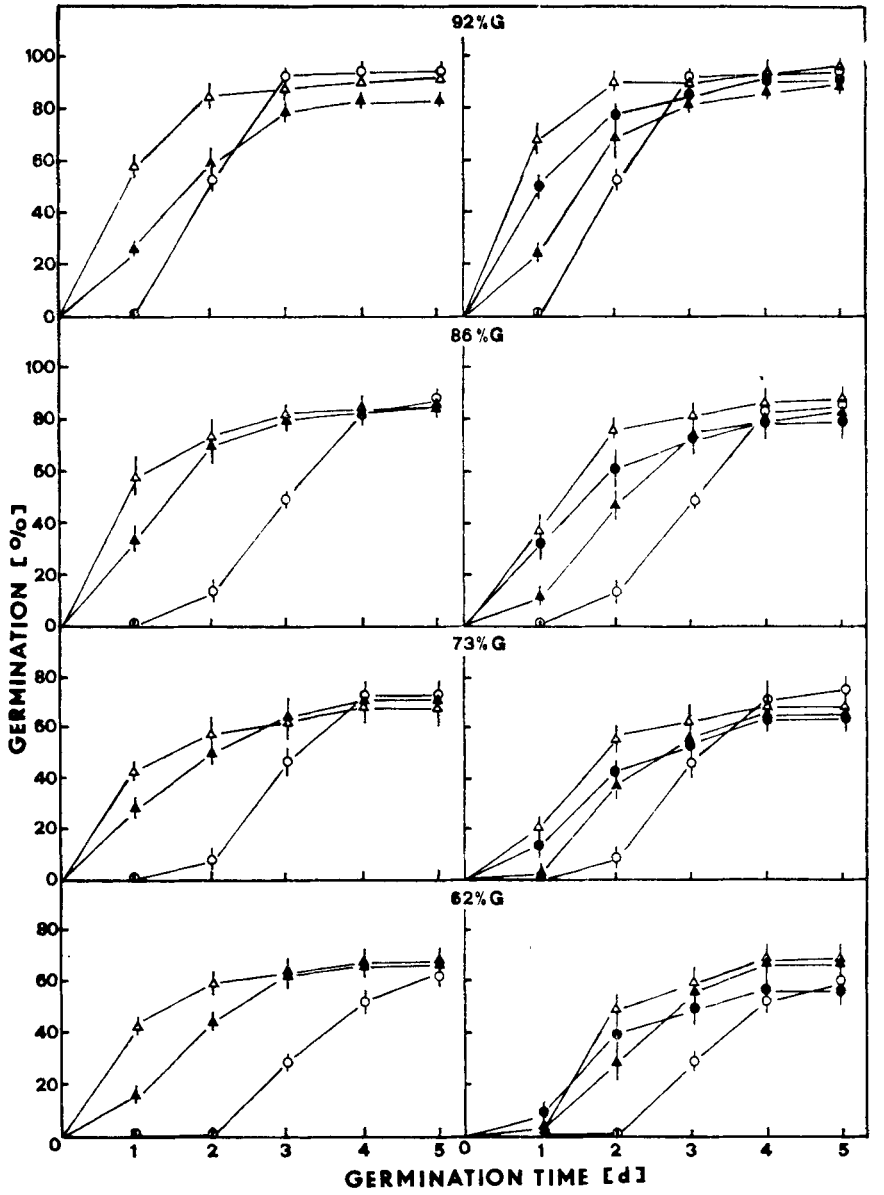

Fig. 1. Time course of germination of primed wheat seed lots at different viability levels in distilled water at 20 °C following incubation at −0.38 MPa and −1MPA for 2 d at 20 °C (on the left) and at 0 MPa, −0.38 MPa and −1 MPa for 4 d at 5 °C (on the right). ○ unprimed seeds; ● 0 MPa primed seeds; △ −0.38 MPa primed seeds; ▲ −1 MPa primed seeds. Vertical bars represent standard error of the mean ($\bar{x}$ for $n = 5$).

imbibition and later on tended to overlap when germination proceeded. When the seeds were treated with other combinations of temperature and duration of treatment (for 4 days at 5 °C) the slope of germination curves changed progressively from the first day of imbibition in relation to viability of seed lot. Gain in germination rate in aged seeds was different from that of

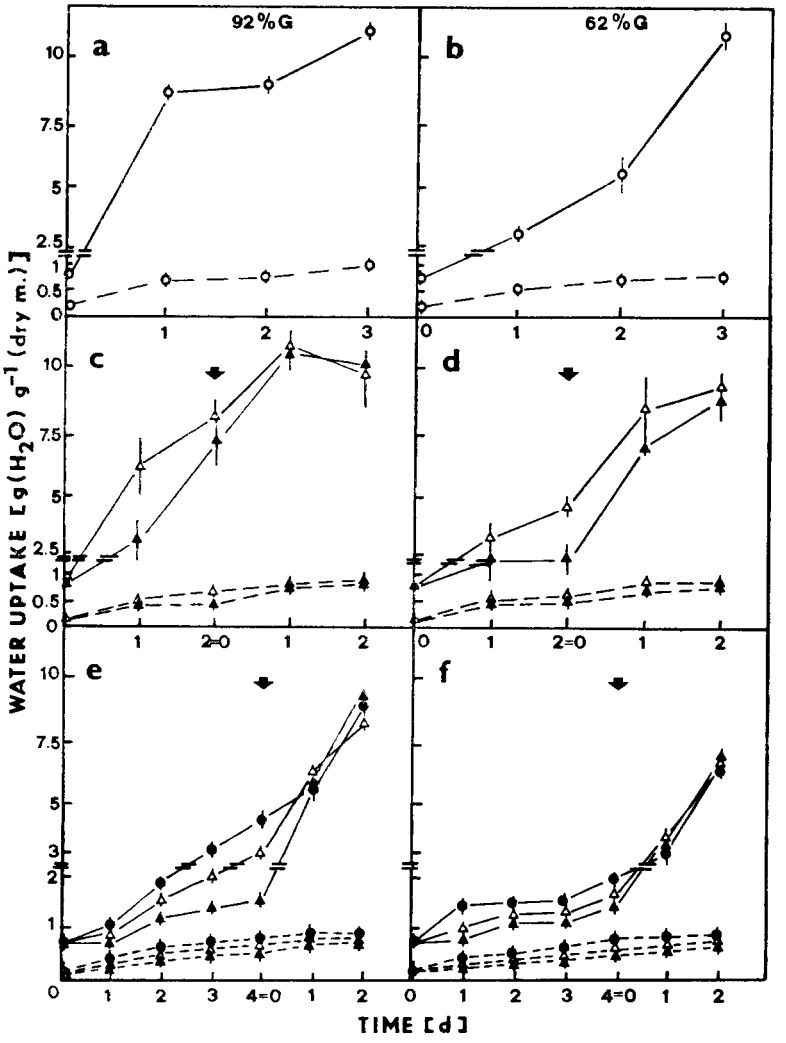

Fig. 2. Time course of water uptake by the embryo (or seedling) (full line) and the rest of grai (dashed line) dissected from wheat seeds at 92% G (on the left) and at 62% G (on the right during osmopriming treatment and following germination in distilled water. Fig. 2a and Fig. 2b are referred to unprimed seeds; Fig. 2c and Fig. 2d are referred to priming conditions at −0.38 MPa and −1 MPa for 2 d at 20 °C; Fig. 2e and Fig. 2f are referred to priming conditions at 0 MPa, −0.38 MPa and −1 MPa for 4 d at 5 °C. The arrows indicate the time limit between PEG 6000 exposure and start of imbibition in distilled water.

Symbols as in Fig. 1. Vertical bars represent standard error of the mean ($\bar{x}$ for $n = 6$).

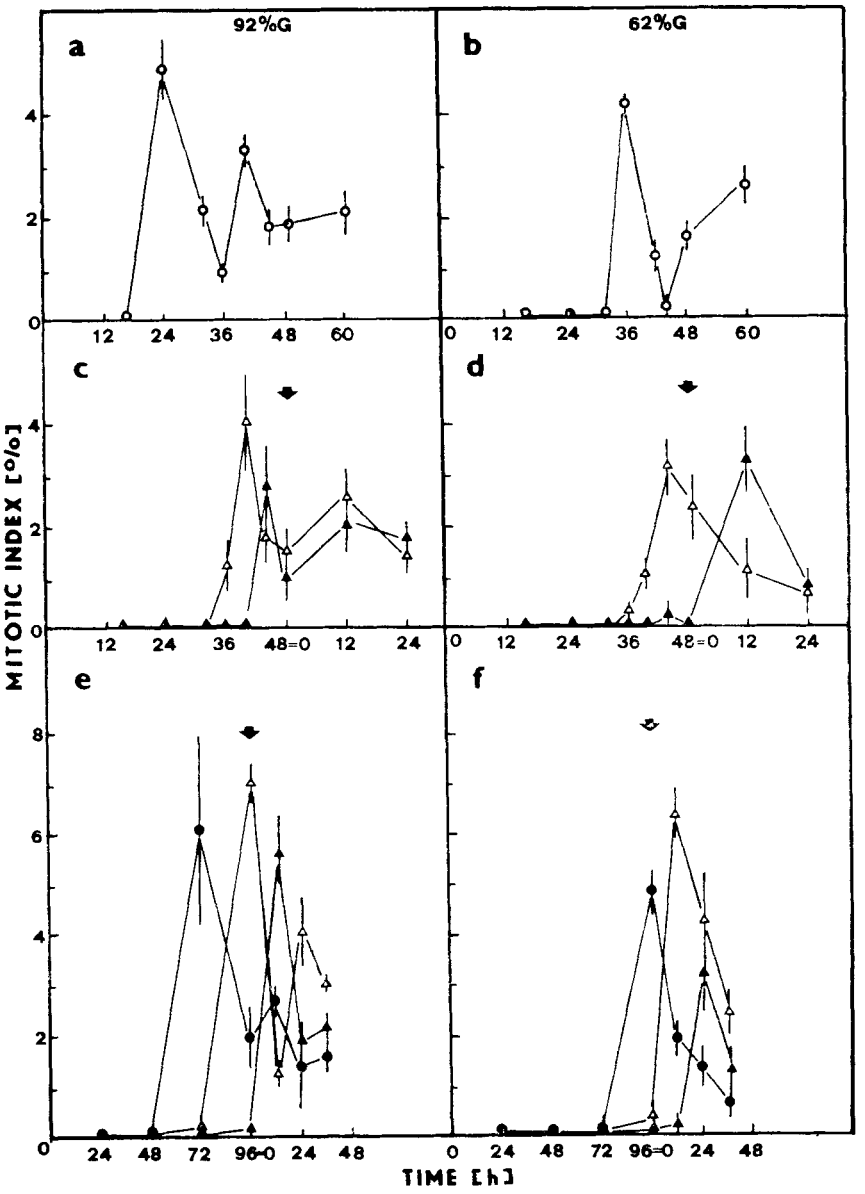

Fig. 3. Time course of mitotic index values in primary root cells of embryo (or seedling) dissected from wheat seeds at 92% G (on the left) and 62% G (on the right) during osmopriming treatment and following germination in distilled water. Fig. 3a and Fig. 3b are referred to unprimed seeds; Fig. 3c and Fig. 3d are referred to priming conditions at −0.38 MPa and −1 MPa for 2 d at 20 °C; Fig. 3e and Fig. 3f are referred to priming conditions at 0 MPa, −0.38 MPa and −1 MPa for 4 d at 5 °C. The arrows indicate the time limit between PEG 6000 exposure and start of imbibition in distilled water.

Symbols as in Fig. 1. Vertical bars represent standard error of the mean ($\bar{x}$ for $n = 5$).

the first treatment and decreased in time progressively with −0.38 MPa, 0 bars and −1 MPa of applied osmotic potentials.

The relationship between the rapidity of germination and water stress induced by decreasing PEG potential was investigated by studying the pattern of water uptake in the embryo (or seedling) and in the rest of the seed. In 92% G and 62 % G seed lots the pattern of water absorption, reported on dry weight basis, showed a progressive decrease in the rate of hydration following the changes of osmopriming conditions in both morphological parts of the seed (Fig. 2). The embryo was more affected by water stress and its ability to regain level of water comparable with that of germinating embryo of untreated seeds may indicate the limit of tolerance of this part of seed to PEG priming treatment. Combinations of unfavourable conditions of treatment could be inefficient to regain satisfactory water status when imbibition proceeds in distilled water. Imperfect germination or lowering of germination rate and final percentage in seeds exposed for a more prolonged time to osmotic potentials lower than −1 MPa confirmed this trend (data not shown).

The reactivation of mitotic activity in embryo cells during PEG treatment was also investigated. No mitotic figures were observed up to 16 h and 32 h of germination in 92% G and 62% G unprimed seeds, respectively (Fig. 3). At 24 h and 36 h the mitotic index value reached the maximum and then decreased to minimum value at 36 h and 44 h in both seed lots, respectively. When seeds were exposed to osmopriming treatment for 2 days at 20 °C the first peak of mitosis shifted progressively with the decrease of osmotic potential. The same trend was observed for seeds exposed to the second kind of treatment (for 4 days at 5 °C) and the delay of the first maximum of mitotic activity at 5 °C was higher than that at 20 °C.

DISCUSSION

Aged seed lots, having a partial germination capacity because of prolonged and unfavourable storage conditions, encounter several difficulties in reaching uniform germination and fast field emergence after sowing. With the correct application of osmopriming treatment on this kind of seed, it is possible to synchronize germination and avoid an unfavourable germination performance. HEYDECKER and COOLBEAR (1977) discussed the effects of osmopriming treatment within a seed lot in terms of seed population configuration change stimulating a higher number of seeds to begin germination before unprimed seeds. A seed sample may consist of one or more sub-groups, each having a different probability of germinating in a given time unit and therefore changing the shape of the corresponding pattern of germination (BOULD and ABBOL 1981). In aged seed lots, the sub-group configuration affects more critically the onset and subsequent rate of germination as aging proceeds. After the pretreatment by osmopriming at 20 °C, modifications to the original shape of the germination curves are appreciable. Priming treatment at 5 °C does not induce similar changes when seed viability decreases. The observed effects, due to different priming temperatures, can be related to different physiological changes which gradually affect the number of sub-groups and thereby increase the probability of synchronous germination.

The control of mitosis reactivation in embryos represents a stimulating method in the more effective investigation of the mechanisms leading to cell division and following cell expansion. Two physiological aspects may be more interesting: those connected with DNA metabolism up to the first S-phase and those connected with initiation of cell division and cell enlargement. Reactivation of enzyme activities involved in DNA repair and replication as well as mitosis resumption are well correlated with the pattern of the first DNA synthesis in aged wheat embryos (DELL' AQUILA *et al.* 1980). The delay of the first cell divisions may result in an increased synthetic capacity during PEG treatment which is less effective at low temperature, leading to a more favourable metabolic balance at the start of germination in treated seeds in comparison with untreated seeds, as also reported in PEG treated tomato seeds by COOLBEAR and GRIERSON (1979). On the other hand reactivation of mitotic activity in embryo cells is a very important event connected with subsequent cellular expansion (HABER and LUIPPOLD 1960a). These two processes do not begin simultaneously in seeds held in mannitol solutions, the first event occurring earlier than the second one (HABER and LUIPPOLD 1960b). Experiments reported in this paper indicate that a slight reduction in water uptake in seeds exposed to decreasing osmotica potentials and temperatures retards cell division and visible germination, perhaps by a block preventing the initiation of cellular expansion. Osmoregulation may also act *via* the cell membrane (MARRÈ 1977) and this may be a relevant factor controlling membrane functionality during the pretreatment and subsequent rapid hydration phase, as suggested by HEGARTY (1978). Mitotic reactivation could be taken as a limiting event to establish the maximum extension of priming treatment conditions preventing germination and stimulating the following germination in distilled water.

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BOOK REVIEW

GOTTSCHALK, W., WOLFF, G.: INDUCED MUTATIONS IN PLANT BREEDING. — Springer Verlag, Berlin—Heidelberg—New York—Tokyo 1983. 238 pp. Cloth DM 98,—; approx. US $ 40.50.

Plant breeding essentially consists of two phases: generation of genetic variability and selection of useful genotypes. Induced mutations contribute primarily by increasing genetic variability. According to the data presented by the International Atomic Energy Agency, more than 500 varieties developed by means of induced mutations, have been officially released. The aim of this book appearing in the series: Monographs on Theoretical and Applied Genetics as Volume 7, is to review the present data of and prospects for applied mutagenesis in plant breeding. The introductory chapters deal with the methods for inducing mutations. It is interesting that 91.4% of the released sexually propagated varieties have been induced by ionizing radiation; in vegetatively propagated crops and ornamentals even 99.2% — the remaining varieties were induced by chemicals. Further chapters cover the chimerical structure of M_1 plants and the selection value of mutant genes. The core of the book focuses on the various types of induced mutants: alteration of the shoot system, flower shape and color, leaf mutants, mutations affecting the root system, flowering and ripening times, disease, drought and shattering resistance and the alteration of seed storage substances. Special chapter are devoted to the utilization of mutants in crossbreeding and mutations in vegetatively propagated crops and ornamentals. The pleiotropic gene action and the penetrance behavior of mutant genes as negative factors in mutation breeding are also discussed.

As has been true in previous volumes in this series, Monographs of Theoretical and Applied Genetics, continues to provide detailed authoritative reviews of subjects of profound interest. This book, serving as an excellent reference source (more than 1 200 references) will be of interest to plant breeders and geneticists.

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