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LEVELS OF PROLINE, GLYCINEBETAINE, POTASSIUM AND MAGNESIUM IN THE GERMINATING WHEAT SEEDS

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Abstract

Changes in levels of proline, glycinebetaine, potassium and magnesium in different tissues of germinating wheat seeds were studied. The tissues of the quiescent wheat grains contained proline, glycinebetaine and inorganic ions. During germination the proline levels increased many fold. The aleurone tissue and the embryo contained large quantities of glycinebetaine and glycinebetaine levels observed in the embryo/seedling arose mainly from the aleurone tissue during germination. The comparative contribution of glycinebetaine and proline to tissue osmotic potential is discussed.

Introduction

It has been proposed that glycinebetaine and proline act as cytoplasmic osmoticum in many halophytes and semitolerant glycophytes (Stewart & Lee, 1974; Wyn Jones, *et al.* 1977). An attempt was made to apply this hypothesis to the partially dehydrated but viable aleurone and embryo tissues of quiescent and germinating wheat grains which may be considered as especially adapted to water stress. Work on seeds was carried out to test whether glycinebetaine is accumulated in dehydrated but viable tissues of plants in view of previous evidence associating glycinebetaine accumulation with low sap water potential (Wyn Jones *et al.*, 1977). Glycinebetaine and proline levels were compared in the tissues in view of the claims that proline is involved in drought resistance (Singh *et al.*, 1973b). Besides attempt was made to obtain evidences on the hormonal control of glycinebetaine and proline synthesis in wheat seeds.

Materials and Methods

Seeds of wheat (*Triticum vulgare,* cv Elsom) were used in batches of 2.3g (40 seeds). The sterilization, incubation and dissection of the plant material was similar to that described by Chittenden *et al.*, (1978). The extraction of glycinebetaine and proline was done according to the method described by Storey & Wyn Jones (1977). Potassium and magnesium were extracted from separate samples of plant material by dry ashing.

Proline was determined by the method of Singh *et al.* (1973a). Glycinebetaine was determined using method described by Storey & Wyn Jones (1977). Potassium and magnesium were measured by flame emission/absorption spectrophotometry using

Pye-Unicam Spectrophotometer. Each experiment was conducted twice and each analysis was also repeated thus each value recorded is the average from several determinations.

Results and Discussion

Proline

The levels of proline in the aleurone tissue, the starchy endosperm and the embryo tissue increased slowly during the first two days of germination and then showed a rapid increase up to the fourth day (Fig. 1). Thereafter the proline level declined in the starchy endosperm, remained constant in the embryo, and continued to increase in the aleurone tissue. The transport of total free amino acids from the starchy endosperm to the embryo has been observed in wheat (Chittenden, *et. al.*, 1978). However, the results of this study revealed no conclusive evidence for any such transport of proline.

To test whether or not the increase in the levels of proline in the aleurone storage tissue during germination, is controlled by GA (Gibberellic Acid) secreted by the embryo (Chittenden *et. al.*, 1978), experiments with de-embryo grains were conducted. In the control endosperms (endosperm incubated in water), there was only a small increase in proline levels (Fig 2) as compared to that observed in germinated grains (Fig. 1). The addition of GA to incubating endosperms caused an increase in proline levels very similar to that found in the germinating grains. Thus, the production of proline in the aleurone tissue of the germinating grains is possibly related to the action of a natural gibberellin secreted by the embryo.

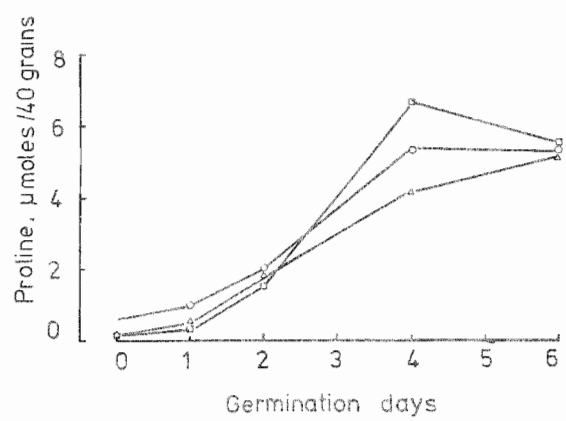

Fig. 1 Time course showing proline levels in the embryo (o) the aleurone (△) and the starchy endosperm (□) tissues during germination of wheat grains. (C.V. $< 5\%$).

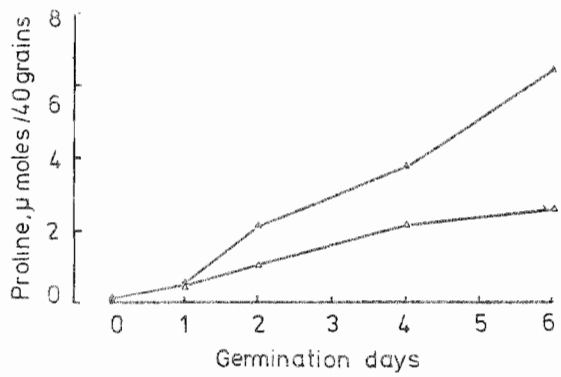

Fig. 2. Time course showing proline levels in the aleurone tissue from endosperm halves incubated in water (△) and 1 mol m^{-3} GA (▲). (C.V. $<5\%$).

Clycinebetaine

The levels of glycinebetaine in the starchy endosperm were low and changed little during germination (Fig. 3). The highest level of glycinebetaine was observed in the aleurone tissue of the quiescent grain which increased by 30% during the first day of germination and then declined progressively up to the sixth day. In the embryo tissue, glycinebetaine levels increased rapidly between the first and the fourth day of germination and this period of rapid increase coincided with the period of rapid decline in gly-

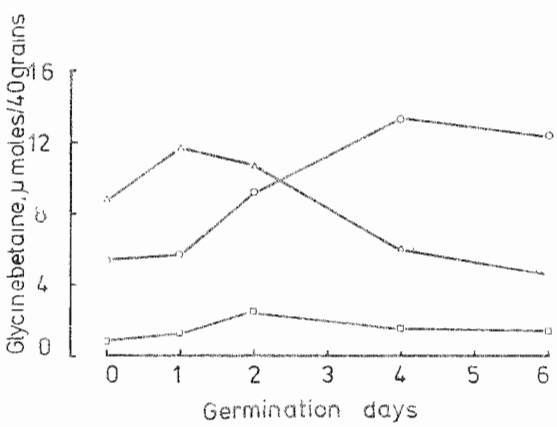

Fig. 3. Time course showing glycinebetaine levels in the embryo (○), the aleurone (△) and the starchy endosperm (□) tissues during germination of wheat grains. (C.V. $<10\%$).

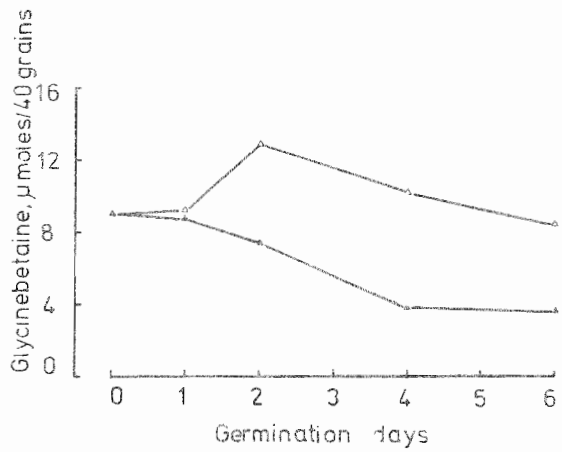

Fig. 4. Time course showing glycinebetaine levels in the aleurone tissue from endosperm halves incubated in water (△) and 1 m mol m^{-3} GA (▲). (C.V $<10\%$).

cinebetaine levels in the aleurone tissue. These results suggest that glycinebetaine levels observed in the embryo tissue arose mainly from the aleurone tissue during germination. Glycinebetaine levels in the aleurone tissue of the control endosperms (embryoless seeds) increased after the first day (Fig. 4) whereas in germinating grain this increase occurred earlier (between 0-1 days), indicating the integrity of the seeds to be necessary for the early rise in the level of glycinebetaine of the aleurone layer. The level of glycinebetaine in aleurone tissue of the endosperm incubated in water was maximum on the second day and then declined. Application of GA decreased the glycinebetaine level after the first day of germination (Fig. 4). Thus, removal of the embryo caused a dramatic increase and application of GA induced a large decrease in the level of glycinebetaine. The results suggest that changes in glycinebetaine levels of aleurone tissue from germinating wheat grains are related to the action of a natural gibberellin secreted by the embryo. A possible GA-induced increase in release of glycinebetaine from the aleurone tissue can be envisaged, while the embryo would act as a sink for glycinebetaine in whose absence higher glycinegetaine levels in the aleurone are maintained.

The aleurone and embryo tissues of wheat seeds being partly dehydrated, may be considered as especially adapted to water stress and in this context it is interesting to note a high concentrations of glycinebetaine found in these tissues (Fig. 3) in contrast to the starchy endosperm (a dead tissue). On tissue water basis, the glycinebetaine concentrations in the aleurone and embryo tissues of the quiescent grains were *ca.* 400 and 200 mol m^{-3} respectively denoting a major contribution of the glycinebetaine to the tissue osmotic potential and possibly cytoplasmic stability (Pollard & Wyn Jones, 1978). However, in contrast to glycinebetaine, proline contribution was small as evidenced by very low levels of proline accumulated in these tissues of the quiescent grains.

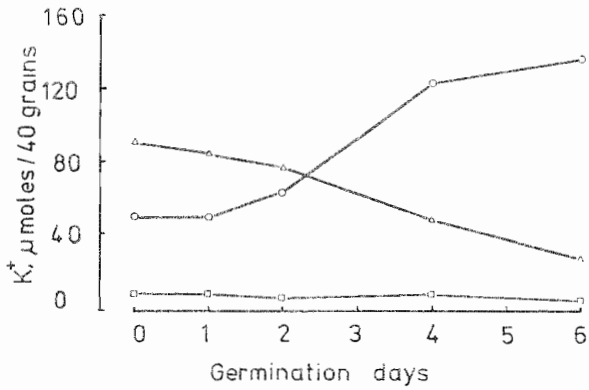

Fig. 5 Time course showing K^+ levels in the embryo (o), the aleurone (△) and the starchy endosperm (□) tissues during germination of wheat grains. (C.V. < 3%).

Potassium and Magnesium

K^+ content of aleurone tissue of quiescent grains was high and declined during germination (Fig. 5). In contrast, the K^+ level in the embryo tissue increased after the first day of germination and the most rapid increase occurred between 2 and 4 days of germination. K^+ content of the starchy endosperm, however, showed little variation during the period of germination. The changes in Mg^+_2 contents of the aleurone and embryo tissues during germination (Fig. 6) followed the trend similar to that for K

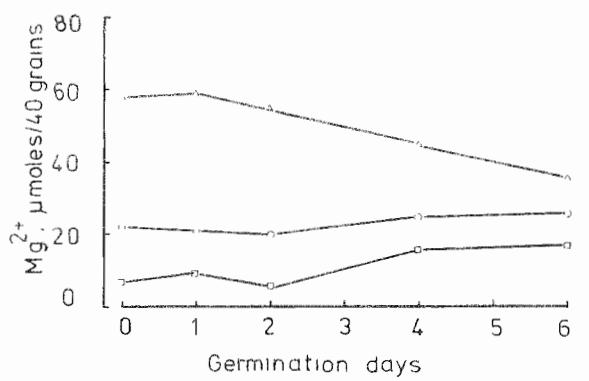

Fig. 6. Time course showing Mg^+_2 levels in the embryo (o), the aleurone (△) and the starchy endosperm (□) tissues during germination of wheat grains. (C.V. < 5%).

contents, with the exception that the rates of changes were much slower. Mg_2^+ content of the starchy endosperm was low in the quiescnet grains but, unlike the K content, increased after the 2nd day of germination. These results indicate that K contents of aleurone tissue are translocated almost completely to the growing embryo (Okamoto, 1962). The relative slow movement of Mg_2^+ from the aleurone to the embryo tissue is in line with the data on leaf distribution of these ions (Ahmad & Wyn Jones, 1982).

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