

Gibberellin-Like Activity in Cotyledons and Embryonic Axes during Pea Seed Germination

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Abstract. Endogenous gibberellin-like activity was determined in dry pea seeds (*Pisum sativum* cv. Bórdli), in cotyledons and axes of germinating pea seeds and also in excised cotyledons and axes. During the first two days of pea seed germination, neither the embryonic axes nor the cotyledons show a mutual influence on gibberellin activity, but this appears after 72–96 h of germination. The gibberellin-like activity in cotyledons and axes of germinating seeds increased during the same period, but it decreased in isolated axes and excised cotyledons.

Several reports have pointed out the influence of embryonic axes on development and maintenance of hydrolytic activity in pea or bean cotyledons (CHIN *et al.* 1972, YOMO and SRINIVASAN 1973, BRYANT and HACZYCKI 1976). It has also been suggested that in cereal endosperm gibberellins and/or cytokinins are involved in regulation of the activity of various hydrolytic enzymes, including amylase and proteases, and that the effect of the embryo can be replaced by exogenous phytohormones (JACOBSEN and VARNER 1967, EASTWOOD *et al.* 1969, HARVEY and OAKS 1974, THOMAS *et al.* 1978). Much less is known about the control of reserve food mobilization in the cotyledons of legumes. When the axes have been removed from the seeds, exogenous gibberellic acid does not restore the hydrolytic activity of cotyledons to values similar to those in intact seeds (VARNER *et al.* 1963, LOCKER and ILAN 1975, HOTA and ISRAELSTAM 1978). GARCIA LUIS and GUARDIOLA (1978) suggested that gibberellins influenced the metabolism of cotyledons indirectly by stimulating the growth of axes.

In the present investigation the activity of endogenous GA-like substances was studied in dry seeds of *Pisum sativum* as well as during germination, and also the incubation of excised cotyledons and axes. This study is part of a project concerning the role of endogenous growth substances and embryonic axes in seed germination.

MATERIAL AND METHODS

In the present study the content of GA-like substances was determined in pea seeds (*Pisum sativum* L. cv. Bördi), using the methods described earlier (PARYS *et al.* 1983, 1984). The activity of endogenous gibberellins was investigated in the dry seeds, and in excised cotyledons and embryonic axes during 96 h of germination or incubation.

The procedure for extraction and determination of GA-like activities in plant tissue was identical to that described previously (ROMANOWSKA *et al.* 1987).

Experiments were repeated 6–9 times. The results shown are the averages of these repetitions $\pm$ S.D.

RESULTS

Gibberellin-like activity in cotyledons of germinating seeds increased about twofold during 48 h of germination as compared with dry seeds, then decreased (72 h) and increased again (Fig. 1).

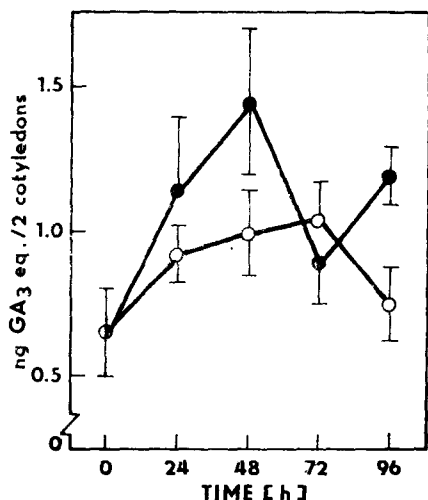


Fig. 1. Gibberellin-like activity in attached (●) and excised (○) cotyledons of peas during germination or incubation.

In the excised cotyledons this activity did not change markedly during 72 h of incubation, after which it decreased slightly to a level about 40 % lower than in the attached cotyledons. Gibberellin-like activity in attached axes increased slightly until 24 h of germination; then it increased rapidly during the remainder of the period (Fig. 2a). In the excised axes activity increased slightly to 24 h of incubation after which it remained at a similar level. Fig. 2b shows the gibberellin-like activity of the axes calculated on a unit fresh matter basis. The activity in excised and attached axes increased until 24 h of germination or incubation, and then decreased. After 72 h of germination the activity in attached axes was about half that of excised axes and decreased thereafter whereas in excised axes it remained constant. The activity in attached axes decreased after 48 h of germination (Fig. 2b) because the fresh matter of the axes sharply increased. The fresh matter

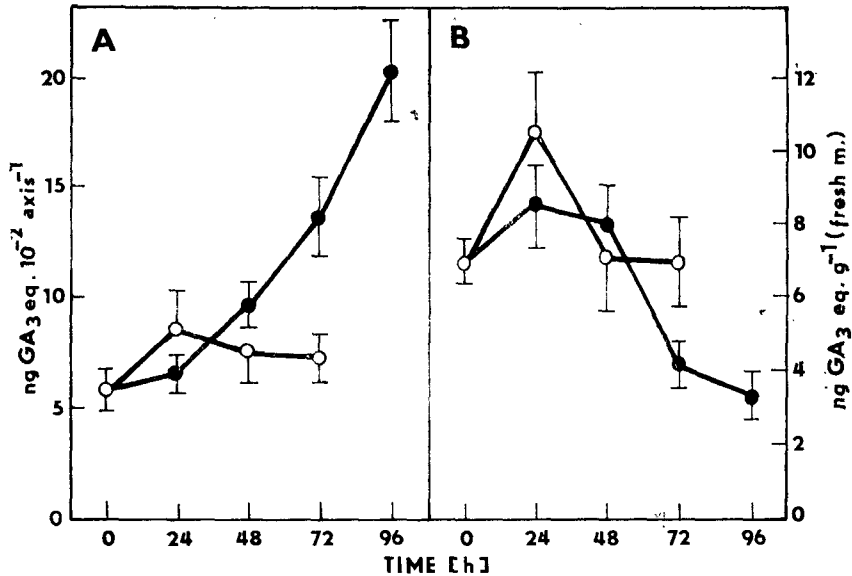


Fig. 2. Gibberellin-like activity in attached (●) and excised (○) axes of peas during germination or incubation. Expressed per axis (A) or on the basis of fresh matter (B).

of excised and attached axes up to 24 h of incubation or germination was similar, but thereafter it rapidly increased in attached axes (Fig. 3).

DISCUSSION

Changes in activity of gibberellin-like substances during development and maturation of pea and bean seeds are well documented (McCOMB 1961, FELIPPE 1967, YAMANE *et al.* 1975). These substances are present in comparative abundance in immature seeds but decrease in amount as the seeds

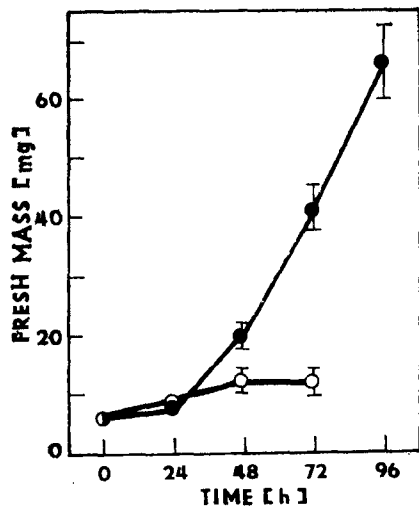


Fig. 3. Fresh matter of attached (●) and excised (○) axes of peas during germination or incubation.

mature (SKENE and CARR 1961, OGAWA 1963). During maturation the gibberellins are converted into bound forms (HIRAGA *et al.* 1974). On the other hand during seed germination the bound GA is hydrolized, releasing active aglycones necessary for seedling growth (BARENDSE *et al.* 1968).

The gibberellin-like activity in dry seeds and during germination observed in the present study is in agreement with the findings of other authors (DALE and FELIPPE 1968). It is a question whether or not the presence of embryonic axes has an effect on gibberellin activity in pea cotyledons during the first days of germination. Our results (Fig. 2a,b) show that up to 48h of seed germination the embryonic axes had no effect on the activity of gibberellins in cotyledons. This is consistent with the data of DALE (1969) who noted that gibberellin activity in bean cotyledons was not dependent upon the presence of embryonic axes to 96h of germination. These results indicate that during the first days of germination the release of bound gibberellins is not dependent on the presence of the embryonic axes. BARENDSE *et al.* (1968) reported that during the first 2–3 days of germination the growth of pea seedlings is independent of *de novo* GA synthesis. During the same period, free gibberellins are released from the bound forms. Rapid decrease in gibberellin-like activity in cotyledons after 48h of germination was connected with intensive growth of the embryonic axes (Fig. 3). Moreover, the matter of excised axes was nearly the same as in attached axes up to 48h of incubation. This indicates that during the first days of germination the axis growth depends upon reserve materials stored in the embryo. Probably up to that time the gibberellins released in the cotyledons were not transported to the axes.

Our data suggest that slightly increased levels of gibberellin-like activity in axes to 48h of germination or incubation is a result of release of gibberellins from bound forms. After 48h of germination we observed in intact axes a large increase in gibberellin-like activity compared with excised axes (Fig. 2a). This might be the result of the transport of phytohormones from the cotyledons to the growing axes. From the results presented above we conclude that, during the first two days of pea seed germination, neither embryonic axes nor cotyledons jointly influence gibberellin activity; the influence of these organs appears after 2 days of germination. The isolated axes and cotyledons at that time showed considerable decrease in gibberellin-like activity. According to some reports regarding the influence of gibberellins on the activity of hydrolytic enzymes in cotyledons of pea and bean seeds (LOCKER and ILAN 1975, GARCIA-LUIS and GUARDIOLA 1978), we assume that these phytohormones play an important role in the integration of metabolism during seed germination.

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