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Two NAD-dependent Alcohol Dehydrogenases (E.C. 1.1.1.1) in Callus Cultures of Wheat, Rye and Triticale

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Summary. Two NAD-dependent alcohol dehydrogenases ADH-1 and ADH-2, under independent genetic control of genes designated as *Adh-1* and *Adh-2* located on chromosomes 4A, 4B and 4D, have been reported in *aestivum* wheat (Hart 1980). Only ADH-1 is expressed in developing seeds, dry seeds, pollen and germinating seedlings. ADH-2 can be induced in seedling roots or shoots under conditions of partial anaerobiosis or by certain chemicals. Expression of ADH-1 and ADH-2 isoenzymes was investigated in undifferentiated calli from *aestivum* and *durum* wheats, rye, triticale and also in in vitro regenerated roots and leaves from *aestivum* cultures. Wheat callus cultures originating from seed, mature and immature embryos, mesocotyl and root, as well as cultures grown on media containing different supplements did not show any variation in the overall expression of ADH-1 or ADH-2, although differences in the band intensities were observed. The callus isoenzyme pattern was similar to that observed in roots under anaerobic conditions. Both ADH-1 and ADH-2 were expressed in in vitro regenerated roots but were absent in regenerated leaves. Expression of ADH-1 and ADH-2 in wheat calli seems to be related to the type of differentiation.

Key words: Wheat – Rye – Triticale – Callus cultures – Alcohol dehydrogenase – Isoenzymes

Introduction

NAD-dependent alcohol dehydrogenase in wheat and its related species belonging to the subtribe Triticinae is known to be a dimeric enzyme (Hart 1969; Mitra and Bhatia 1971; Jaaska and Jaaska 1980). After the electrophoresis of dry and germinating seed or embryo extracts of bread wheat, three bands can be observed which result from a random association of monomers, the structural gene(s) of which have been located on chromosomal arms 4Ap, 4Bp and 4Dp. These code respectively for the α, β and δ monomers. Subunit composition of the three bands, as suggested by Hart (1970), is $\alpha\alpha$; $\alpha\beta$, $\alpha\delta$ and $\beta\beta$, $\delta\delta$, $\beta\delta$. A similar three band pattern is observed in most tetraploid wheats belonging to the *Triticum turgidum* group. However, only two types of monomers α and β are present. Genetic variants have been reported in tetraploid wheats (Hart 1969; Suseelan 1980). Rye shows only one major band, the structural gene for which has been located on short arm of chromosome IV (after Riley and Chapman 1958) of rye King II (Mahajan 1975). On seed germination this enzyme starts disappearing in 4–5 day-old seedlings and is not observed in leaves. Enzyme bands similar to those found in dry seeds are observed in extracts from pollen grains and developing kernels about ten days after pollination.

Another inducible NAD-dpendent ADH has been reported in wheat (Jaaska 1976; Jaaska and Jaaska 1980; Hart 1980) which is induced in the roots and shoots under conditions of partial anaerobiosis. This ADH enzyme has been designated as ADH-I (inducible) by Jaaska and Jaaska (1980) and ADH-2 by Hart (1980). The genetic control of this (ADH-2) is independent of the ADH described previously which is now designated as ADH-1 (Hart 1980). ADH-2 is also a dimeric enzyme and structural genes have been located on chromosomes 4A, 4B and 4D. It is reported that monomers of ADH-1 and ADH-2 reassociate to form active dimers which result in seven ADH bands (Jaaska and Jaaska 1980; Hart 1980). Further, it has been shown that both ADH-1 and ADH-2 isoenzymes, normally not seen in 3–4 day-old seedling leaves, can be induced by anaerobiosis either by submerging them in water for 24 h or by keeping them in a nitrogen or argon atmosphere.

Thus, with the available genetic information, it was thought desirable to use this system to investigate further the induction of ADH enzymes in wheat callus cultures. Initial studies have shown that unlike leaves, callus cultures have both ADH-1 and ADH-2 isoenzymes. This prompted us to further investigate the two ADH enzymes in wheat, rye and triticale callus cultures grown on different media, and also of different origin.

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Materials and Methods

Hexaploid wheat (*Triticum aestivum* (L.), Thell.) cv. 'Kalyan Sona', tetraploid wheat (*T. turgidum* L. ssp. *durum*) cv. 'Bijaga Yellow', rye (*Secale cereale* L.) and triticale (*Triticum aestivosecale* MacKey) were used as source material. Callus cultures were raised from seeds, mature and immature embryos, roots and mesocotyls. To initiate seed callus cultures, seeds were surface-sterilized in 70% ethanol (v/v) for 30 sec, in 0.1% $HgCl_2$ (w/v) for 5 min and then rinsed several times with sterile distilled water. They were then placed on a basical medium containing the mineral salts of Murashige and Skoog (1962), vitamins of Lin and Staba (1961) and 2% sucrose. The medium was supplemented with one of the followings: p-chlorophenoxyacetic acid (PCPA), α-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4-5-trichlorophenoxyacetic acid (2,4,5-T) or 2,4,5-trichlorophenoxypropionic acid (2,4,5-Cl_3POP) at 5 mg/l. The pH of the medium was adjusted to 5.8 before autoclaving and solidified with 0.6% agar. Callus cultures from immature embryos were induced by culturing embryos from seeds collected three weeks after anthesis. Mature embryo calli were initiated from embryos isolated from seeds pre-soaked in water for 16–20 hours. Mature embryo calli induced on medium containing 2,4,5-Cl_3POP were transferred to different media supplemented with coconut milk (CM), benzyladenine (BA), naphthoxyacetic acid (NOA), indolebutyric acid (IBA), indoleacetic acid (IAA), NAA and PCPA, either alone or in combinations. Calli from mesocotyl, leaf and root were obtained from 6 day-old seedlings grown under sterile conditions. Cultures were grown under continuous illumination (950 lux) at 25 ± 2 °C. Friable calli from seeds were obtained in media supplemented with 2,4-D, 2,4,5-T and 2,4,5- Cl_3POP, while NAA and PCPA containing media produced calli accompanied by rooting. Calli used for isoenzyme studies were harvested after 30–40 days of incubation during the second passage and homogenized with cold 0.01 M Na-pyrophosphate buffer pH 9.5 in a chilled mortar. In all extractions, the ratio of tissue to buffer was 1:2 (w/v). The slurry was centrifuged at 4 °C for 30 min at 12,000 × g. The supernatant (400 µl) was used for electrophoresis in standard polyacrylamide gels (7%) at constant (400) volts, essentially following the procedure of Davis (1964). ADH bands were visualized by incubating the gels in the reaction mixture, the composition of which has been given by Brewer (1970).

Results

Hexaploid wheat calli established from seeds cultured on various media had seven ADH bands (Fig. 1a), which are numbered as 1 to 7 (Fig. 2a). On co-electrophoresis of dry seed and callus extracts, bands 5, 6 and 7 of the calli overlapped with the three bands observed in dry seed extracts (Figs. 1b, 2b) and hence these bands must belong to the ADH-1 enzyme. Bands 1, 2 and 3 belong to the ADH-2 and band 4 is probably a hybrid band formed as a result of the reassociation of the ADH-1 and ADH-2 monomers. This pattern was identical to that observed in roots under anaerobic conditions. Callus cultures grown on media containing different supplements such as NAA, PCPA, 2,4-D, 2,4,5-T and 2,4,5-Cl_3POP at varying concentrations did not show any variation in the isoenzyme pattern. Callus cultures obtained from mesocotyl, root and mature and immature embryos when electrophoresed under similar conditions also showed seven bands (Figs. 1e, 2e). However, band 1 was usually very faint or not seen at all in extracts from the calli of mature and immature embryos (Figs. 1c, 2c). In vitro produced roots also exhibited seven band pattern (Figs. 1d, 2d). ADH bands were absent in in vitro regenerated leaves.

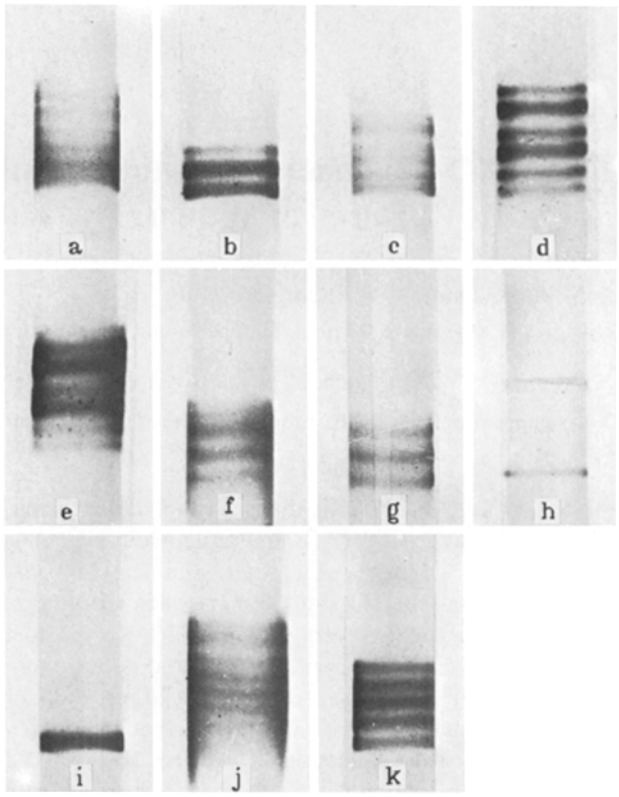

Fig. 1a–k. ADH zymograms of bread wheat Kalyan Sona seed callus **(a)**, dry seed **(b)**, embryo callus **(c)**, root callus **(d)**, and regenerated root **(e)**; tetraploid wheat 'Bijaga Yellow' seed callus **(f)**, and dry seed **(g)**; rye seed callus **(h)**, and dry seed **(i)**, and triticale seed callus **(j)**, and dry seed **(k)**

Tetraploid wheat callus cultures from cultivar 'Bijaga Yellow' showed a 5-band pattern (Figs. 1f, 2f). Dry seeds of this cultivar had a different ADH-1 pattern (Figs. 1g, 2g) from those of bread wheat and other durum wheat cultivars. It is similar to the variant reported by Hart (1969) and Suseelan (1980) in *T. dicoccum*. Bands 3, 5 and 7 co-electrophoresed with the three bands in seed extracts of this cultivar.

Rye callus obtained from seeds and embryos on basal medium supplemented with 2,4,5-Cl_3POP showed two widely separated bands (Figs. 1h, 2h), one of which corresponded to band 3 (ADH-2) observed in wheat calli. The other band co-electrophoresed with ADH-1 (band 9) observed in dry seed extracts of rye (Figs. 1i,

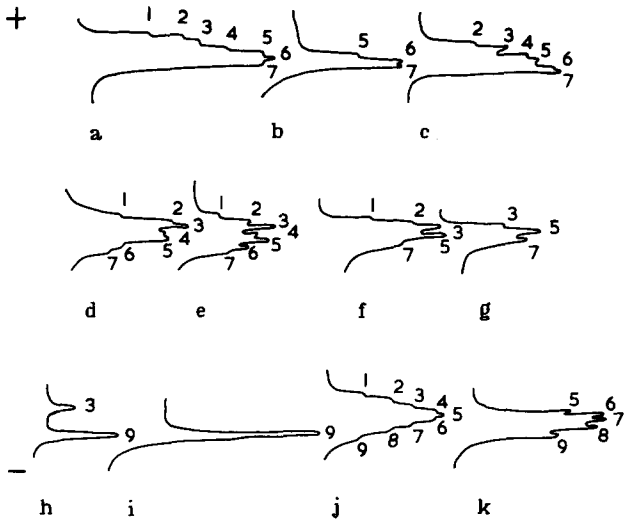

Fig. 2a–k. Densitometer recordings of ADH zymograms of bread wheat 'Kalyan Sona' seed callus **(a)**, dry seed **(b)**, embryo callus **(c)**, root callus **(d)**, and regenerated root **(e)**; tetraploid wheat 'Bijaga Yellow' seed callus **(f)**, and dry seed **(g)**; rye seed callus **(h)**, and dry seed **(i)**; and triticale seed callus **(j)**, and dry seed **(k)**

2i). Rye roots also showed the pattern similar to that of rye callus. No hybrid band between rye ADH-1 and ADH-2 was observed.

Triticale callus cultures obtained from seeds and embryos showed 9 bands (Figs. 1j, 2j), of which bands 1–7 and 5–9 were similar to those observed in wheat calli and triticale dry seeds (Figs. 1k, 2k), respectively. Band 9 co-electrophoresed with the rye dry seed band. Band 8, as reported previously (Irani and Bhatia 1972) is a hybrid band formed by association of rye and wheat ADH monomers.

Densitometer tracings (Fig. 2) revealed that band intensities observed in callus cultures were, in general, different from those of the seed extracts. Relative band intensities also differ in calli of different origin.

Discussion

Expression of both ADH-1 and ADH-2 isoenzymes in the wheat, rye and triticale callus cultures observed was similar to that obtained after induction of both ADH-1 and ADH-2 in seedling roots under anaerobic conditions. Both ADH-1 and ADH-2 isoenzymes have been expressed in some maize callus cultures (Freeling et al. 1976) which were friable and classified as class II by the authors. The other type of maize callus cultures which were parenchymatous, non-friable and nodular expressed only ADH-1.

Three bands in wheat, one in rye and five in *triticale* callus extracts overlapped with the ADH-1 bands observed in dry seed, pollen or developing grains. ADH-1 or ADH-2 has not been observed in mature leaves or other aerial plant parts. Further, the leaves obtained from regenerated seedlings growing in the medium did not show either ADH-1 or ADH-2 bands similar to those found in normal leaves from seed grown plants, while roots originating from calli showed both ADH-1 and ADH-2 (Figs. 1e, 2e). This indicates that the expression of *Adh-1* and *Adh-2* genes in differentiating wheat calli is related to the type of differentiation. Such a system may therefore provide clues to the manipulation of expression of the *Adh* genes by using chemicals that induce differentiation or de-differentiation under in vitro conditions. Variation in the band intensities in calli originating from embryo, mesocotyl and root of hexaploid wheat points to differential expression or regulation of *Adh-1* or *Adh-2* gene(s) on the chromosomes of A, B and D genomes.

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