Activity of Alpha-Amylase in Induced Mutants of Barley

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Abstract
Selection of lines with high levels of alpha-amylase activity is one of the major goals of malting barley breeders. The alpha-amylase activity, assayed in the seed and seedlings of induced mutants of barley, was found to be on the increasing side in most of the mutants compared to that of control. Further, enzymatic activity showed an increase in the imbibed seed compared to fresh seed and four days old seedlings. The mutants with enhanced activity of alpha-amylase may be developed into superior cultivars of barley for malt industry.
Key words: Alpha amylase- Induced mutants- Barley.

Introduction

Mutation induction has become an established tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits. Progress in breeding of crop plants depends upon selection of favourable combination of characters available in the population. Starch, the chief source of carbohydrate in human diets, forms the major energy reserve in cereal grains. In cereal endosperms it is present in the form of discrete, insoluble particles called starch granules. Alpha-amylase is of prime importance in initial stages of starch degradation whether this occurs in naturally germinating cereal grains in the soil or under controlled industrial processing such as malting and brewing (Macgregor, 1983). The action pattern of this enzyme is of wide importance and interest. Selection of lines with high levels of alpha-amylase activity is one of the major goals of malting barley breeders. Alpha-amylase is formed by hormone controlled *de novo* synthesis in grains but the precise roles played by aleurone, seed-coat and embryo tissues have yet to be clarified. The enzyme is heterogeneous and some components may be more efficient than others in hydrolyzing starch granules. Barley mutants with changes in the activity of alpha-amylase may be of help in better understanding of the problem.

Materials and Methods

An experiment on the estimation of alpha-amylase activity in barley involving six induced mutants along with their parental control was conducted. The M_6 seeds of the 6 mutants viz. dwarf, semi-dwarf, early maturing, semi-dwarf with early maturity, lax spike and chlorina types, isolated from the barley cultivar 'K169' were used along with their control. Alpha-amylase activity was assayed following Filner and Varner (1967) in fresh and imbibed seeds and also 4 days old seedlings. The seedlings were grown in trays in a growth chamber under controlled light and temperature conditions. The materials were homogenized in chilled grinding medium and centrifuged. The supernatant was made up to a final volume of 10 ml with chilled distilled water and this serves as crude enzyme preparation. 1.0 ml of crude enzyme extract prepared as above was added to 1.0 ml substrate (0.15% potato starch, 0.2mM $CaCl_2$ in boiling extract) incubated for 10 min at room temperature and the reaction was quenched by addition of 3 ml iodine reagent (0.6% iodine, 6% KI in water). 1.0 ml of this was diluted to 50 ml with 0.5N HCl. The A_{620} of

the reaction mixture (1.0 ml enzyme added to a mixture of 1.0 ml substrate and 3 ml iodine reagent) was read against blank (containing no substrate). The total activity was estimated against a standard and expressed as mg substrate degraded per min per gram fresh tissue.

Results and Discussion

The alpha-amylase activity in imbibed seed was highest compared to fresh seed and 4 days old seedlings (Table 1). All mutants of 'K169' showed increased activity of the enzyme alpha-amylase over that of control. In imbibed seed, the alpha-amylase activity was highest in the seed of chlorina mutant (12.54 mg/g) followed by lax spike mutant (12.31 mg/g), early maturing (11.99 mg/g) and semi-dwarf early maturing mutant (11.79 mg/g) while in the dwarf mutant (11.00 mg/g) it was almost similar to that of control ('K169'; 10.96 mg/g fw) (Table 1).

In the fresh seed of mutants and control, wide variation was observed in the amount of enzyme with the mutants exhibiting activity on either side of the parental control. In the mutants, the activity of alpha-amylase in the fresh seed ranged from 5.34 mg/g in the dwarf mutant to 7.97 mg/g in the semi-dwarf mutant compared to 6.61 mg/g in the control (Table 1).

Alpha-amylase activity is an important quality factor in malting barley. Selection of lines with high levels of alpha-amylase activity is one of the major goals of malting barley breeders. Research in physiology and molecular genetics has shown that alpha-amylase activity in barley is influenced by allelic differences at several loci located on chromosomes 1 and 6 (Brown and Jacobsen, 1982) and hormone levels within the seed (Jacobsen and Chandler, 1987).

Alpha-amylase activity was on the increasing side in most of the mutants of the present study compared to their control. Further, the enzyme activity showed an increase in the imbibed seed compared to fresh seed and four days old seedlings. This is expected as the alpha-amylase is synthesized *de novo* in the cells of aleurone layer in response to gibberellins secreted by embryo upon germination (Ho, 1979) while in the seedling the enzyme activity declines because of fall in the substrate.

In all mutants of 'K169', the enzyme activity was found to be higher over that of control. Hater and Riggs (1973) reported significant genotypic variation for alpha- amylase activity and observed that expression was not highly affected by environment. However, Sekiguchi *et al*. (1984) concluded that selection for alpha-amylase activity could be effective despite a large environmental effect. The mutants with enhanced amylase activity can profitably be utilized in the breeding programmes for development of superior barley varieties for malt industry.

Table 1. Alpha-amylase activity in fresh seed, imbibed seed and 4 days old seedlings of induced barley mutants.

Mutant/Control	Alpha amylase activity					
	Fresh seed		Imbibed seed		4 days old seedlings	
	mg/g	% control	mg/g	% control	mg/g	% control
'K169' Control	6.61±0.42	--	10.96±0.04	--	8.19±0.01	--
Dwarf	5.34**±0.01	80.78	11.00±0.04	100.36	9.79*±0.01	119.53
Semi-dwarf	7.97**±0.06	120.57	11.79±0.04	107.57	9.95**±0.03	121.48
Semi-dwarf early maturing	6.54±0.15	98.94	11.92±0.04	108.75	7.88±0.04	96.21
Early maturing	6.27±0.01	94.85	11.99±0.01	109.39	9.17*±0.04	111.96
Lax spike	7.54*±0.08	114.06	12.31*±0.04	112.31	9.63*±0.10	117.58
Chlorina	6.69±0.09	101.21	12.54*±0.01	114.41	9.83*±0.07	114.52

± : S E value; * p: 0.05, **P : 0.01

References

Brown, A.H.D. and J.V. Jacobsen, 1982: Genetic basis and natural variation of alpha- amylase in barley. Genet. Res. (Cambridge) 40, 315-324.

Filner, P. and J.E. Varner, 1967: A test for de novo synthesis of enzymes: density labeling with H_2O^{18} of barley α-amylase induced by gibberellic acid. Biochemistry 58, 1520-1526.

Hayter, A.M. and T.J. Riggs, 1973: Environmental and varietal differences in diastatic power and four associated characteristics of spring barley. J. Agric. Sci (Cambridge) 80, 297-302.

Ho, T., 1979: Hormonal control of enzyme formation in barley aleurone layers. In : Molecular Biology of Plants (I. Rubenstein *et al*., Eds.). Academic Press, New York.

Jacobsen, J.V. and P.M. Chandler, 1987: Gibberellin and abscisic acid in germinating cereals. Pages 164-193 in "The Biochemistry and Physiology of Gibberellins" (ed. Davies, P.G.). Kluwer Acad. Publ., Boston.

MacGregor, A.W., 1983: Cereal alpha-amylases: Synthesis and action pattern. In: Seed Proteins (J. Daussant; J. Mosse and J. Vaughan, eds.). Annual Proceedings of the Phytochemical Society of Europe, 20, 1-34. Academic Press, London.

Sekiguchi, T.; G. Takeda and K. Kawaguchi, 1984: Quality test for the breeding of malting barley in relation to alpha-amylase activity. Jpn. J. Breed. 43, 263-272.