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Effect of Autopolyploidy on Quantity and Quality of Protein in Barley

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Summary. The range and mean protein content of autotetraploids of high-lysine Notch-2 mutants of barley were consistently higher than the diploids in C_3 and C_4 generations of colchicine treated seeds. Amino acid analysis of whole grain meal of diploid Notch-2 and one strain of its autotetraploid revealed differences in the amino acid composition. The proportion of albumin in the diploid and the autotetraploid Notch-2 was higher by 21% and 45% respectively, in comparison to the parent 'N.P. 113', whereas the glutelin fraction was significantly higher in the autotetraploid. The autotetraploid, with increased glutelin and decreased prolamin, showed no increase in lysine. It is possible that the recessive high-lysine gene may be lacking dosage effect, resulting in no increase in lysine in the autotetraploid, whereas protein content, a polygenically controlled trait, is enhanced due to genome duplication.

Key words: Autopolyploidy – Protein estimation – Barley

Introduction

Subsequent to the isolation of opaque-2 and floury-2 mutants in maize by Mertz, Bates and Nelson in 1964, studies were initiated in the attempt to improve the quality of crop plants. Nutritional improvement of plants by timely applications of nitrogen fertilizers has been recognized as one method for improving quality but due to the rise in price of fertilizers, it is not a feasible approach from an economic point of view. Genetic upgrading of protein quality has now been accepted as the only alternate method. Genetic manipulation, either by induced mutation or substitution of chromosomes from allied genera, has been advocated and adopted in order to achieve improvement of protein quality and quantity in cereal crops. The duplicating of genomes and the attendant changes in the nutritional aspect are, in comparison, less investigated. An unequal segregation of chromosomes in autopolyploids could affect fertility and yield, and might be a reason in not employing this technique for improving the nutritional quality of crop plants. In addition to building up genetic stocks, it is possible to study the effect of ploidy on protein quality. With this objective, the present study with a high-protein high-lysine mutant and its induced autotetraploids of barley was undertaken. The observations recorded are communicated.

Material and Methods

Seeds of the high-protein high-lysine Notch-2 mutant, isolated from barley variety 'N.P. 113' (2n = 14) following mutagenic treatment with ethylmethane sulfonate (Bansal 1970), were used in the present study for colchicine treatments and isolation of autotetraploids. Quantification of crude protein (N X 6.25) in seeds of Notch-2 mutants and its autotetraploids were done using a Technicon Nitrogen Autoanalyser.

For determining the soluble fractions, a modified method of Osborne (1924) was followed. Dried mature grains ground to 80 mesh flour were defatted with a moisture free mixture of chloroform, methanol and acetone (2:1:1). Using water as a solvent, the albumin fraction was first extracted; followed by the globulin fraction using 5% (W/V) sodium chloride; the prolamine fraction with 70% aqueous ethanol; and glutelin with 0.1M sodium hydroxide. Four extractions of each sequential fraction were made at time intervals of three hours, two hours, two hours of shaking the material in respective solvents and lastly after a one hour wash and shaking in water. After each shaking, the contents were centrifuged at 15,000 rpm for 20 minutes at 4°C in a Sorvall RC_2-B Automatic Refrigerated Centrifuge and the four supernatants of individual fractions pooled for final analysis. After dehydration of the fractions, the protein content was determined using a Technicon Nitrogen Autoanalyser.

Estimation of amino acids were done according to Weidner and Eggum (1966) on Beckman Amino Acid Analyser Model 120 C at the Department of Animal Physiology and Chemistry, National Institute of Animal Sciences, Copenhagen, Denmark.

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Table 1. Protein content in C_3 and C_4 seeds of autotetraploids and diploid Notch-2

Generation	Chromosomal constitution	No. of plants analysed	Protein (%)		Protein/grain (mg)		10 grain weigth (g)	
			Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.
C_3	2n	16	19.06-20.31	19.92±0.31	6.40- 7.60	6.99±0.09	0.32-0.38	0.35±0.02
	4n	18	23.49-27.81	25.76±0.26	7.73-11.29	9.52±0.23	0.32-0.43	0.37±0.02
C_4	2n	4	16.25-18.12	17.18±0.44	5.14- 6.18	5.61±0.25	0.30-0.34	0.33±0.01
	4n	21	20.62-31.87	26.19±0.56	6.80-11.51	8.94±0.26	0.26-0.39	0.34±0.01

Results

Whole seed meal protein of diploid Notch-2 mutant and its autotetraploids were analysed in C_3 and C_4 generations and the data are given in Table 1. The range and mean protein content in autotetraploids were consistently higher than in the diploids for both generations. The amount of protein per grain was higher in autotetraploids whereas grain weight showed no significant differences when compared to the diploids.

Amino acid analysis of whole grain meal of diploid Notch-2 and one strain of its autotetraploids revealed differences in the amino acid composition (Table 2). In contrast to the diploid, the tetraploid had reduced amounts of essential (EAA^s) and non-essential ($NEAA^s$) amino acids except for glutamic acid which showed an increase in the tetraploid by 44%. The milligram amounts of limiting amino acids lysine, threonine, isoleucine and leucine per gram N in the diploid and the tetraploid were 1042.4 and 878.7, respectively. However, the amounts of these limiting, as well as other essential and non-essential, amino acids showed a reverse trend when calculated on the basis of unit weight of flour (g/100g flour).

Table 2. Amino acid composition of whole seed protein of Notch-2 mutant and its autotetraploid

Amino acid	Notch-2 (2n)		Notch-2 (4n)	
	g/16g N	g/100g flour	g/16g N	g/100g flour
Asp	6.61	1.18	6.03	1.49
Thre	2.85	0.51	2.38	0.59
Ser	2.86	0.51	2.45	0.60
Glu	22.24	3.99	32.17	7.94
Pro	9.23	1.65	9.58	2.36
Gly	4.31	0.77	3.40	0.84
Ala	4.36	0.78	3.48	0.86
Val	5.09	0.91	4.25	1.05
Ileu	3.60	0.64	3.15	0.78
Leu	6.49	1.16	5.47	1.35
Tyr	3.15	0.56	2.60	0.64
Phe	4.63	0.81	4.50	1.11
Lys	*3.74*	0.67	3.06	0.75
His	2.08	0.37	1.77	0.44
NH3	2.07	0.37	2.36	0.58
Arg	4.92	0.88	4.52	1.11
Meth	1.86	0.33	1.72	0.42
Cys	2.15	0.38	1.99	0.49
N%	2.87		3.95	

Protein fractionation data of Notch-2 mutant, the parental variety 'N.P. 113' and autotetraploids are presented in Table 3. Protein loss due to defatting of flour was negligible. The proportion of albumin in the diploid and the autotetraploid Notch-2 was higher by 21 and 45% respectively in comparison to the parent 'N.P. 113'. Though the globulin content of 'N.P. 113' and Notch-2 was similar, in the tetraploids it was 40% less. Prolamin content in the diploid and tetraploid Notch-2 was comparable, whereas in the parental strain 'N.P. 113', a higher value (22%) was recorded. The major fraction, glutelin, was significantly larger in the autotetraploids compared to the parental variety and the Notch-2 mutant, both of which had similar values (Fig. 1).

Discussion

In recent years, attempts have been made to improve cereal protein by suitable agronomic practices (Johnson et al. 1973) and by the isolation of mutants, both spontaneous (Mertz et al. 1964; Nelson et al. 1965; Munck et al. 1970) and induced (Bansal 1970; Doll et al. 1974; Mohan and Axtell 1975). Chromosome manipulation techniques have also been utilized in improving the protein content. Law et al. (1978) showed that in wheat, substitution of chromosome 2D by 2M of *Aegilops comosa* enhanced the grain protein without affecting grain yield.

The present study clearly suggests that protein content can be increased by doubling the chromosome number. A 52% increase in protein could be achieved by inducing autotetraploidy. Analysis of C_3 and C_4 generations revealed the enhanced protein values to be comparatively stable (Table 1). Since no significant differences in grain weight between the diploids and tetraploids were recorded, it is evident that the protein per grain was higher

Table 3. Grain protein fractions (%) in NP113, diploid and autotetraploid Notch-2

Genotype	Crude protein (%) in defatted sample	Albumin	Globulin	Prolamin	Glutelin	Residue	Extractability
'NP113'	12.5	15.5	16.2	17.3	32.7	18.3	81.6
Notch-2(2n)	16.9	18.9	16.1	15.9	32.9	16.3	83.7
Notch-2(4n)	23.7	22.6	11.5	14.1	40.0	11.9	88.1

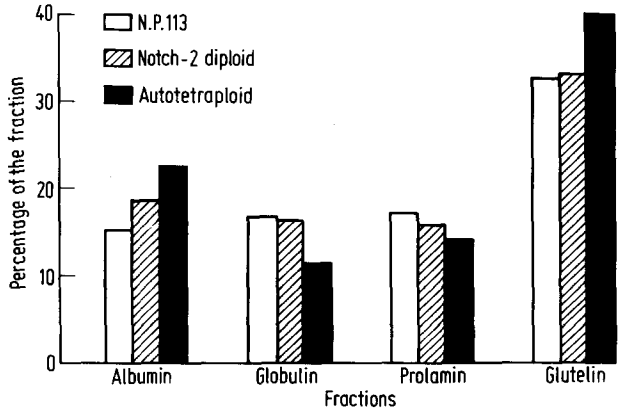

Fig. 1. Proportions of different soluble fractions in seed protein of 'N.P. 113' diploid Notch-2 and autotetraploid

in autotetraploids than in diploids. The higher protein in autotetraploids with no increase in grain weight compared to the diploid Notch-2 suggests that this increase in the protein is at the expense of starch synthesis. Increased level of protein content by 53% following induced autotetraploidy in barley has also been reported by Manzuk and Barsukov (1974).

While the increase in protein in diploid Notch-2 compared to the parental strain 'N.P. 113' is attributable to an enhanced albumin fraction (Bansal et al. 1977), the increase observed in the tetraploid is due to increased albumin and glutelin fractions (Fig. 1). Even though the amounts of all amino acids per unit weight of flour in autotetraploids were more, values for amino acids per unit weight of protein varied. An increase in the non-essential amino acid glutamic acid alone was observed whereas the values for all the remaining amino acids showed a downward trend (Table 2). An alteration of the proportion of protein fractions in cereals has been advocated as one of the methods for achieving changes in amino acid composition. Nelson (1969) has suggested that mutations which reduce the concentration of prolamin and allow increased synthesis of other fractions alter the amino acid composition in cereals. In the diploid Notch-2 mutant, an increase in the albumin and a reduction in the prolamin fractions have resulted in an increase in total protein and lysine. In comparison, in the autotetraploids, a reduction in the globulin and prolamin fractions and an increase in the albumin and glutelin fractions enhanced only total protein values but not lysine, which showed a reduced value. In opaque-2 mutants of maize, a higher proportion of lysine in the glutelin fraction has been reported (Nelson 1969). It is surprising that the autotetraploids with increased glutelin and reduced prolamin and globulin fractions showed no increase in lysine. In wheat, the studies of Johnson et al. (1978) have shown that protein differences above 15% have little or no effect on lysine level. It is possible that a similar restraint is operative in autotetraploid barley also. Alternatively, the recessive high lysine gene (Karlsson 1972) may be lacking dosage effects, resulting in no increase in lysine in autotetraploids whereas protein content, a polygenically controlled trait, is enhanced due to genome duplication. All high lysine mutants so far isolated are in the diploid species and none have been found in polyploid species.

Reduction in spike fertility has been associated with increased protein content (Fröst and Ellerström 1965). However, Manzuk and Barsukov (1974) have been able to achieve 93% spike fertility and an increased level of protein content by 53% in autotetraploid barley. In the autotetraploids utilized in the present study the range in spike fertility was 25-59% and may be contributory to higher values of protein. Studies on the correlation of spike fertility and protein content are in progress and attempts are being made to isolate high protein high-fertility autotetraploid strains.

Literature

Bansal, H.C.: A new mutant induced in barley. Curr. Sci. 39, 494 (1970)

Bansal, H.C.; Srivastava, K.N.; Eggum, B.O.; Mehta, S.L.: Nutritional evaluation of high protein genotypes of barley. J. Sci. Food Agric. 28, 157-160 (1977)

Doll, H.; Koie, B.; Eggum, B.O.: Induced high lysine mutants in barley. Rad. Bot. 14, 73-80 (1974)

Fröst, S.; Ellerström, S.: Protein content and fertility in tetraploid rye. Hereditas 54, 119-122 (1965)

Johnson, V.A.; Drier, A.F.; Grabouski, P.H.: Yield and protein responses to nitrogen fertilizer of two winter wheats differing in inherent protein content of their grain. Agron. J. **65**, 259-263 (1973)

Johnson, V.A.; Mattern, P.J.; Wilhelmi, K.D.; Kuhr, S.L.: Genetic improvement of productivity and nutritional quality of wheat. 2nd FAO/SIDA seminar on improvement of nutritional quality in barley and spring wheat. New Delhi 1978 (unpublished)

Karlsson, K.E.: Linkage studies on a gene for high lysine content in Hiproly barley. Barley Genet. Newsl. **2**, 34-36 (1972)

Law, C.N.; Worland, A.J.; Chapman, V.; Miller, T.E.: Homoeologous chromosome transfers into hexaploid wheat and their influence on grain protein amounts. Proc. 8. Congress of Eucarpia 1978 (in press)

Manzuk, V.T.; Barsukov, P.N.: Performance and seed quality of tetraploid barley. Barley Genet. Newsl. **4**, 49-50 (1974)

Mertz, E.T.; Bates, L.S.; Nelson, O.E.: Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science **145**, 279-280 (1964)

Mohan, D.P.; Axtell, J.D.: High lysine mutants of sorghum. FAO/IAEA Mutation Breeding Newsl. **5**, 11 (1975)

Munck, L.; Karlsson, K.E.; Hagberg, A.; Eggum, B.O.: Gene for improved nutritional value in barley seed protein. Science **168**, 985 (1970)

Nelson, O.E.: The modification by mutation of protein quality in maize. pp. 41-54 Proc. symposium on New Approaches to Breeding for Improved Plant Protein. IAEA. 1969

Nelson, O.E.; Mertz, E.T.; Bates, L.S.: Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science **150**, 1469-1470 (1965)

Osborne, T.B.: The vegetable proteins. London: Longmans Green & Co. 1924

Weidner, K.; Eggum, B.O.: Protein hydrolysis: A description of the method used at the Department of Animal Physiology in Copenhagen. Acta agric. Scand. **16**, 115-119 (1966)

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