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EFFECTS OF N-METHYL N-NITROSOUREA (MNU) ON SOME QUANTITATIVE CHARACTERS AND ENDOSPERM STORAGE PROTEINS IN RICE (*ORYZA SATIVA* L.)

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Abstract

The fertilized egg-cells of rice were treated with 1mM N-methyl N-nitrosourea (MNU) to induce mutation. The treatment effects on quantitative characters varied considerably but in most of the cases they were not significant. The MNU effects on storage proteins were quite high. Six groups were identified depending on the staining intensities of the polypeptide bands. The line 121 had low prolamin. The line 175 showed low prolamin content but high glutelin. These two lines can be used as valuable breeding materials for improving protein qualities in rice.

Key words : Rice, MNU, Quantitative characters, Endosperm storage protein.

Introduction

Mutation breeding is one of the effective methods for the improvement of crops. In recent years, it was reported that the effects of mutagenic chemicals in higher plants were related with the specific stages of the DNA synthetic cycle (Yamaguchi, 1972, Yamaguchi and Matsubayashi, 1973; Nishimura and Futsuhara, 1976). Some authors reported that the chemical mutagens are more advantageous than ionizing radiations because of their higher frequency of gene mutations with few chromosomal aberrations (Heiner *et al.*, 1960). Endosperm storage protein of rice is divided into four fractions viz. albumin, globulin, prolamin and glutelin. Glutelin is the major fraction of the total protein in rice (Juliano, 1972). Prolamin fraction is nutritionally inferior, non-digestible and it consists of about 20-25% of the total rice endosperm protein (Ogawa *et al.*, 1987). Prolamin consists of one major polypeptide component with molecular weight (MW) of 13,000 (Tanaka *et al.*, 1980; Ogawa *et al.*, 1987) and glutelin consists of two kinds of components with MW of about 40,000 and 20,000, respectively (Wen and Luthe, 1985, Sarker *et al.*, 1986; Tanaka *et al.*, 1980). For the nutritional improvement of protein in rice the proportion of prolamin should be reduced and glutelin should be increased.

The present research work was undertaken to determine the effects of MNU treatment of

the fertilized egg-cells on quantitative characters and endosperm storage proteins.

Materials and Methods

The fertilized egg-cells of BR2 were treated by dipping the panicles into 1mM MNU solution. One day before the treatment, the opened spikelets were removed. Next day only the spikelets flowered were kept for the treatment and the unopened spikelets were removed.

The panicles containing only fresh fertilized spikelets were immersed into MNU solution for 45 minutes at 24° C under dark condition. The plants were treated at 2 (two) hours interval from the 1st treatment started at 6.00 p.m. In total eight treatments have been given to the plants until 8.00 a.m. of next day. The treated panicles were washed immediately after the treatment was over for 24 hours in running water.

The M_1 populations were grown in Aus season 1989 to multiply the seeds. The M_2 populations were planted along with control in a Completely Randomized Block Design with three replications at the Institute of Postgraduate Studies in Agriculture (IPSA), Gazipur farm during Boro season 1989-90. The data were recorded from 20 plants of each replication. The recorded data were : number of panicles, panicle length (cm), sterility percentage, 100-grain weight (g) , yield per plant (g), grain length and breadth (cm), L/B ratio. Data were subjected to statistical analysis.

Rice powder from a single grain was taken into an eppendorf tube. Seven hundred microliter of sample buffer solution (0.125 M Tris, 4% SDS, 4 M Urea, 20% Glycerin and 5% 2-Mercaptoethanol, P^H 6.8) was added to the rice powder. The sample was mixed well by vortex twice at two hour intervals. The sample was then centrifuged at 15,000 rpm for three minutes.

The extracted proteins were then separated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) using the buffers described by Laemmli (1970) on vertical slab gel containing 14% acrylamide. Five µl of the supernatant was taken for each SDS-PAGE analysis. Electrophoresis was run at room temperature until the bromophenol blue marker reached the bottom of the gel. The gels were stained by 50% methanol, 7% acetic acid and 0.5% coomassie brilliant blue R-250.

Results and Discussion

Effects on quantitative characters

The mean number of panicles varied from 11.73 to 15.13. The highest panicle number was found in control (15.13) followed by treatment IV (14.97). The lowest panicle number was observed in treatment III (11.73). It was observed from the result that the effects of MNU reduced the panicle number but no significant difference was observed among the treatments (Table 1).

The variation in panicle length was 20.40 cm to 24.25 cm. The highest panicle length was found in treatment VII (24.25 cm) followed by treatment VIII (21.66 cm). The control ranked third position (21.45 cm) for panicle length. Only treatment VII showed significant difference from all other treatments at 5% level.

The sterility percentage was generally higher in the treatments than the control except treatment V and VII. The range varied from 28.46 to 35.86%. There was no significant difference in the treatment effects.

For 100-grain weight, all the treatments showed higher grain weight than the control except treatment VI. The range varied from 2.06 (g) to 2.20 (g). It may be noted that due to MNU treatment, the mean grain weight was increased but the treatment effects were not statistically significant. The increase in seed

Table 1. Effects of MNU on some quantitative characters in rice.

Source	Number of panicles	Panicle length (cm)	Sterility %	100-grain weight (g)	Yield/ plant (g)	Grain size		
						length (cm)	breadth (cm)	L/B ratio
BR2	15.13	21.45	31.16	2.09	24.74	0.82	0.25	3.30
	±0.22	±0.36	± 4.27	± 0.03	±2.77			
Treatment I	13.97	21.17	34.01	2.19	22.54	0.86	0.25	3.45
	± 1.28	± 0.61	± 5.41	± 0.02	± 4.18			
Treatment II	14.13	20.81	35.40	2.20	19.78	0.84	0.26	3.24
	± 0.75	±0.43	±0.27	± 0.03	± 0.76			
Treatment III	11.73	20.59	34.37	2.13	15.02	0.84	0.24	3.50
	±1.96	±0.24	±4.09	±0.04	±3.05			
Treatment IV	14.96	20.40	35.86	2.10	22.53	0.83	0.24	3.41
	± 0.78	± 0.34	± 3.89	± 0.02	± 3.67			
Treatment V	13.46	20.40	28.46	2.09	18.24	0.85	0.25	3.40
	±0.42	±0.32	±2.41	±0.01	±1.29			
Treatment VI	14.60	20.82	35.00	2.06	18.11	0.82	0.26	3.17
	±1.92	±0.60	±1.86	±0.04	±0.01			
Treatment VIII	12.20	24.25	30.90	2.14	21.32	0.84	0.26	3.19
	±0.66	±0.64	±1.85	±0.03	±1.48			
Treatment VIII	12.20	21.66	32.70	2.17	21.04	0.82	0.25	3.22
	±0.47	±0.36	±5.46	±0.02	±2.78			
L.S.D. (0.05)	4.82	1.68	15.56	0.13	12.43			
CV.(%)	14 .70	3.32	19.71	2.70	25.59			

weight may be due to some changes at the molecular level because MNU acts as an alkylating agent. MNU acts in many ways during DNA replication by adding ethyl group to guanine or depurination or crosslinkage between the DNA strands.

The yield per plant was highest in control (24.74g) followed by treatment I (22.54g). The range varied from 15.02 g to 24.74 g. The difference of mean yield per plant in the treatments and control were not statistically significant.

The grain length was highest (0.86 cm) in treatment I followed by treatment V (0.85 cm). Control, treatment VI and VIII showed the similar grain length. The variation in grain breadth was very low among the treatments. The range varied from 0.24 to 0.26 cm only. The L/B ratio varied from 3.17 to 3.50. The highest L/B ratio was observed in treatment III.

The overall result indicated that the effects of the MNU on quantitative characters were not consistent. The reasons for not being consistent results may be due to action of MNU at the molecular level. Sometimes it behaves as a base analogue and produces pairing errors or gaps in the DNA chain. Due to pairing errors or gaps in the DNA chain, the ultimate effect may be in both directions.

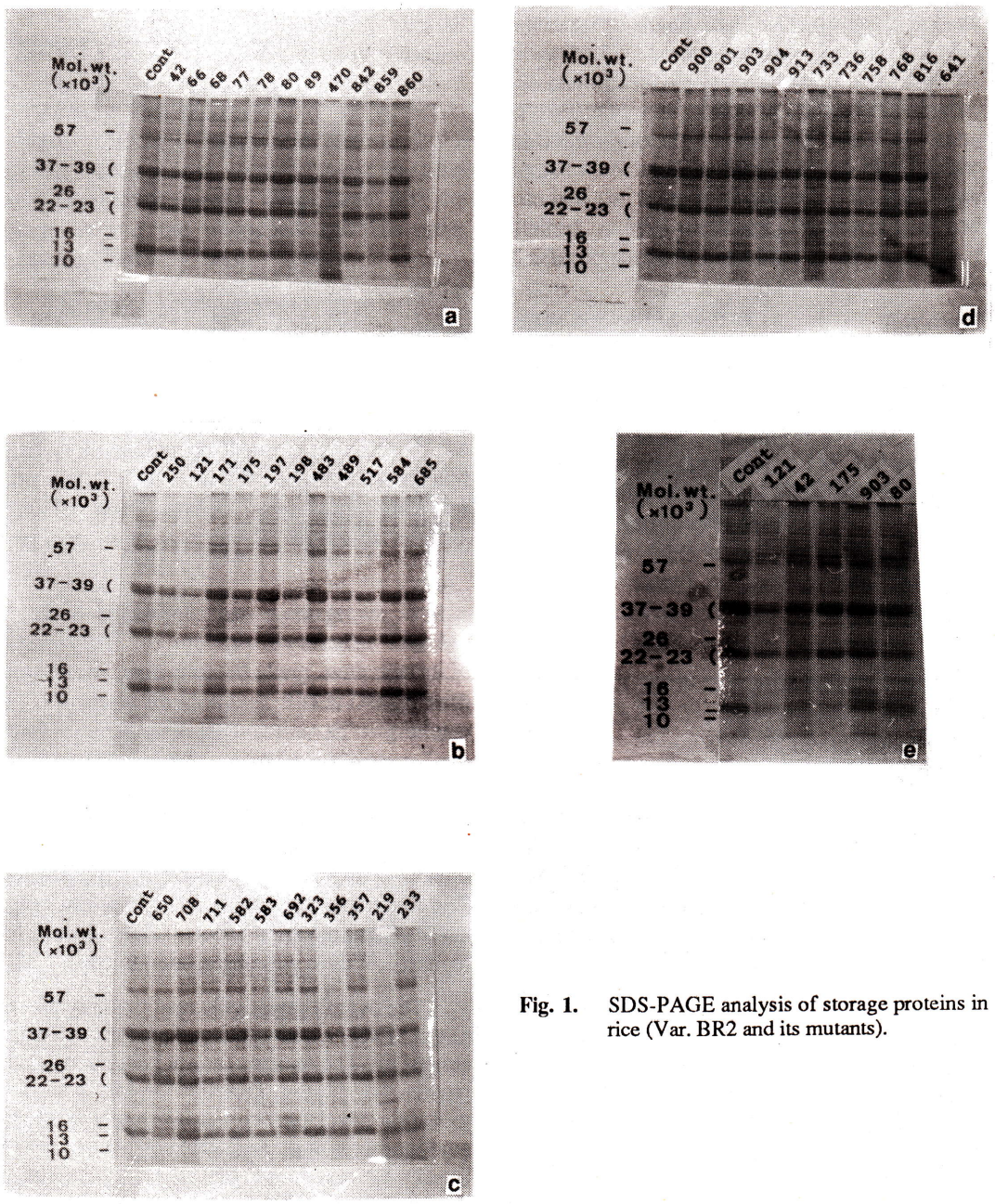

Fig. 1. SDS-PAGE analysis of storage proteins in rice (Var. BR2 and its mutants).

Effects on endosperm storage proteins

Adout 1000 mutants of rice developed from the variety BR2 by treating the fertilized egg-cells with N-methyl-N-nitrosourea (MNU) were screened by SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electro-phoresis) analysis. Among the rice storage proteins, prolamin is nutritionally inferior and non-digestible. It consists of about 20-25% of the total endosperm storage proteins. It comprises one major polypeptide component (13 kDa) and two other minor polypeptide components (10 kDa and 16 kDa). Glutelin comprises two kinds of polypeptide components (20 kDa and 40 kDa). The staining intensity of the polypeptide bands indicate the concentrations of the protein fractions. In Fig. 1a, line 859 showed a considerable variation in the banding patterns in comparison with control. The line 121 showed low staining intensity in 10 kDa, 13a kDa, 13b kDa and 16 kDa polypeptide bands. The line 42 showed low staining intensity in 10 kDa and 16 kDa region. The lines 171, 197, 483, 650, 708 and 903 had dark stain in 10 kDa, 13a,13b kDa, 16, 22-23, 26, 32-39 and 57 kDa region (Fig. 1b, 1c and 1d, respectively). The line 175 showed low staining intensity in 16 kDa region (Fig.1b). The line 250 and 904 showed low staining intensity only in 13b kDa region (Fig.1b and 1d).

Finally, six groups have been identified with distinct polypeptide bands depending on the staining intensities of each polypeptide band (Fig.1e). Kumamaru *et al.* (1988) studied about 3000 mutants of rice (var. Kinmaze) and identified four different types of mutants for rice storage proteins, viz. 1) 13 b polypeptide low (13b-L), 2) 57 kDa polypeptide high (57-H), 3) 10 kDa and 13a polypeptides low (10/13a-L) and 4) 10 kDa and 16 kDa polypeptide high (10/16-H) . Out of six groups identified, only one group (13b-L) was similar with that of Kumamaru *et al.* (1988). In a study of 118 varieties/lines for endosperm storage proteins, Bhowmik *et al.* (1989) also found six different types with distinct polypeptide bands which showed some similarities with the present result.

The line 121 had low prolamin, because the bands 13a and 13b showed very low staining intensities. Line 175 also showed low prolamin but the glutelin content was high. The lines 121 and 175 can be used as valuable breeding material for improving protein quality.

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