6. PAKISTAN

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TECHNICAL REPORT

GENETIC VARIATION OF HIGH MOLECULAR WEIGHT GLUTENINS IN PAKISTANI WHEAT LINES

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INTRODUCTION

Cereal grains are an important source of proteins for human beings and farm animals. Wheat storage proteins can be classified into glutenin, gliadin, albumin and globulin. The glutenin and gliadin are the major components of gluten and are largely responsible for its functionality in bread making and other food system. Both glutenin and gliadin are deposited in storage protein in the developing grain and both are considered to be prolamin.
The glutenin and gliadin have different rheological properties; glutenin imparts elasticity to a bread dough whereas gliadin gives viscosity and extensibility. When glutenin is treated with a reducing agent like 2-mercaptoethanol, it loses its elastic property and dissociates into several subunits that are classified into two groups, i.e., low molecular weight (LMW) and high molecular weight (HMW) glutenin subunits. The storage proteins of wheat flour typically consists of approximately 50% glutenins (40% LMW glutenin subunits and 10 % HMW glutenin subunits).

About 50% of the wheat storage protein is gliadin, a complex mixture of single polypeptides that are crosslinked by interpeptide disulfide bonds (Shewry and Tatham 1989). Reduced glutenin subunits were divided into HMW and LMW subunits (Payne *et al.* 1984). The HMW subunits of glutenin constitute only a small portion (10%) of the storage protein (Payne *et al*. 1984), but they exert a pronounced effect on gluten elasticity and bread making quality of the flour.

The genetics of endosperm protein in bread wheat have been studied intensively by Payne and co-workers, using the method of SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

The polymorphism and electrophoretic properties of HMW subunits in different genotypes of bread wheat has been studied by several workers (Lawrence and Shepherd 1980; Payne *et al*.,1983b; and Shewry *et al*. 1986). The genetic base of the cultivated wheat has led workers to investigate the possibility of utilizing the genetic variation in wild species. The gene pools of wild species are adapted to the range of environments and carry a large reservoir of useful genes(Feldman and Sears, 1981).

Nevo and Payne (1987) studied diversity of HMW glutenin subunits in tetraploid wild progenitor of wheat. They observed that the endosperm of wild emmer wheat (*Triticum dicoccoides*, AABB genome) contains many allelic variants of glutenin storage protein which are not present in bread wheat (AABBDD genome) and could be utilized for improving bread making qualities. Several diploid wheat species have been analyzed for HMW subunit polymorphism based on SDS-PAGE. Lawrence and Shepherd (1980) showed that 28 accessions of *Aegilops squarrosa*, the progenitor of the D genome of bread wheat, all had two HMW subunits with mobilities similar to those of subunits presented in bread wheat.

This research is initiated to determine the genetic variability based on SDS-PAGE electrophoregrams in the wheat material collected from Pakistan and to compare their genetic variation of the HMW subunits of glutenin with standard Japanese and other wheat cultivars.

MATERIALS AND METHODS

Plant material

Eighty eight accessions of Pakistani wheat lines and 21 accessions of Japanese standard cultivars were used for SDS-PAGE (Table 1). The Pakistani wheat lines were collected from Punjab, NWFP and Baluchistan areas by the joint expedition mission of Ministry of Agriculture, Forestry and Fisheries, Japan with collaboration of University of Agriculture, Lyallpur (Faisalabad)- Pakistan during 1974 as shown in Fig.1. After preparation of samples and gels, electrophoresis experiment was conducted.

Sample preparation

The total seed protein was extracted from 40 mg of flour mixed with 1 ml of SDS buffer (1.5M Tris HCl buffer, pH 8.8/0.4% SDS) in an eppendorf microcentrifuge tube. After stirring on vortex mixture, shaking for 2 hours in an automatic shaker, boiling for 3 minutes and centrifuging at 12,000 rpm for 13 minutes, sample was suspended. Just before the electrophoresis, 5 µl of extract was loaded/applied into each well by a microsyringe.

Electrophoresis

The HMW glutenin composition was analyzed through the slab gel type SDS-PAGE following the method of Laemmli, 1970 using the 7.5% polyacrylamide gel . The experiment of electrophoresis was conducted at a current of 16 mA for 8 hours. The gels were carefully removed from the gel glass plates, and all these gels were stained with Coomassie brilliant blue R-250 and then destained with 25% methanol- 7.5% acetic acid solution. After destaining the gels were kept in Gel Drying Processor and were dried for 80 minutes (see, **Appendex** in detail).

RESULTS AND DISCUSSION

Seed protein in common wheat primarily comprises glutenin and gliadin . Glutenin- the least soluble portion of gluten is less amenable to fractionation, but suitable techniques have been devised including SDS-PAGE. This procedure which fractionates the gluten protein as the reduced polypeptide subunits, provides the useful varietal differences.

Eighty eight Pakistani wheat lines were classified into eighteen different groups on the basis of different banding patterns of HMW glutenin subunits as shown in Table 1 and Fig.2.The largest group A consists of about half of land races having HMW glutenin subunits banding pattern as 7+8+2+12 just as Chinese cultivar 'Chinese Spring'(Table 2).The second largest group B of land races having 10 lines showed the characteristic banding pattern as 1+7+8+10. Single subunit 10 on Glu-D1 loci is the characteristic band found in Afghan and Iranian cultivars, because these lines were collected from the locations near to the border of Afghanistan and Iran. The group C of banding pattern 1+7+5+10 belongs to breeding lines. The group D of land races and breeding lines showed the banding pattern 2*+17+18+2+12 which is characteristic of Australian cultivar 'Gabo'.

As a whole on the basis of results of HMW glutenin subunits, Pakistani wheat lines were differentiated into three groups of land races, breeding lines and CIMMYT breeding lines (Table 3). The character of land races was just like Chinese Cultivar 'Chinese Spring' and this group of land races was dominant to the other two groups. Null allele on Glu-A1 loci was found with gene frequencies of 64% whereas 79% of land races showed the subunits 7+8 and 2+12 on Glu-B1 and Glu-D1 loci, respectively. This first group was the collection of Baluchistan Province site III (Sariab, Badozai, Gobark, Aghbarg, Mastung, Kaskkak, Nauhisar and Quetta). All the lines are differentiated as land races and they had no 5+10 subunits on Glu-D1 loci except one Pakistani land race (49P 71-10). Subunits 5+10 and

2+12 are known to be the most effective of HMW glutenin subunits in their effects on dough properties: 5+10 subunits is related to a good rheological property and 2+12 to poor rheological property. The HMW subunits 7+8 are known to have significantly greater beneficial effects on gluten strength and bread making quality than the HMW subunit 20 in land races.

The second group of 30 lines consists of breeding lines. Number of 18 lines of them had shown the subunit 1 on Glu-A1 loci and it was dominant to other subunits on Glu-A1 loci. A number of 13 lines showed the subunits 17+18 on Glu-B1 loci whereas subunit 7 was found in 8 lines and subunits7+8 in 3 lines. Subunit 7 may be associated with greater dough strength and decreased extractability of gluten proteins. In this group subunits 5+10 on Glu-D1 loci was dominant as shown by 17 breeding lines whereas 13 lines showed the subunits 2+12 on Glu-D1 loci. This group was known to be of good bread making quality due to having subunits 5+10 on Glu-D1 loci.

The third group was differentiated as five CIMMYT breeding lines. The two breeding lines 'Mexipak 65' and 'Blue Silver' were collected from Lyallpur (Faisalabad), Punjab which is irrigated area. Other three lines 'Mexipak', 'Sonora 64' and 'Mexico 120' were included as comparison because they have been used as Pakistani breeding materials.

So the breeding lines were mostly collected from Lyallpur (Faisalabad-Punjab) site I and Peshawar, Kohat and Tarnab (N.W.F.P) site II under irrigated conditions, and no land race was found in this area. In breeding lines 5+10 subunits on Glu-D1 loci is common whereas it is rare in the land races.

Compared with Japanese breeding lines, subunits 7+8 on Glu-B1 loci was found in 12 breeding lines, and subunits 2+12 on Glu-D1 loci was found in 7 lines and subunits 2.2+12 on the same loci in 9 lines. Null allele on Glu-A1 loci was found in 10 lines. So Japanese breeding lines had a common banding pattern with such Pakistani land races as presented in Null, 7+8, 2+12 .

On the basis of this characteristic, breeding lines of Lyallpur and Peshawar group might be correlated with the good bread making qualities due to having 5+10 subunits on Glu-D1 loci whereas land races are poor in bread making quality due to having 2+12 subunits. So on the basis of these results we can conclude about Pakistani wheat lines as follows:

CONCLUSIONS

1 . Pakistani land races have Chinese Spring type subunits (Null, 7+8, 2+12). Subunits 7+8 on Glu-B1 loci and 2+12 on Glu-D1 loci might be useful for Pakistani local bread *'Chappati'*.

2. Second group is combination of Australian 'Gabo' type and 'Chinese Spring' type. It has subunits 17+18 on Glu-B1 loci and 2+12 on Glu-D1 loci.
3. Third group of wheat lines grouped as breeding lines having subunits 5+10 on Glu-D1 loci, is CIMMYT breeding line 'Sonora 64' type. It has subunits 17+18 on Glu-B1 loci with subunits 5+10 on Glu-D1 loci. It has known that subunits 5+10 is useful for bread making quality.
4. Compared with Japanese varieties, the first group Pakistani land races have subunits 7+8 on Glu-B1 loci and 2+12 on Glu-D1 loci in common with Japanese old cultivars.
5. A group with single subunit 10 on Glu-D1 loci is characteristic of Afghan and Iranian lines. The reason is that these land races were collected around/near the border of Afghanistan and Iran.This characteristic subunit was found only in land races and not found in CIMMYT and breeding lines.

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LITERATURE CITED

Arakawa,T., Yoshida,M., Morishita.H., Honda. J. and Yonezawa, D. 1977. Relation between aggregation behaviour of glutenin and its polypeptide composition. Agric. Biol. Chem. **41**: 995-1001.

Blakesley, R.W. and Boezi, J. A., 1977. A new staining technique for proteins in polyacrylamide gels using Coomassie Brilliant Blue G 250. Anal Biochem. **82**: 580-582.

Brown,J.W.S., Kemble,R.J., Law, C.N. Flavell, R.B. 1979. Control of endosperm proteins in *Triticum aestivum* (var. Chinese Spring) and *Aegilops umbellata* by homoeologous group 1 chromosomes . Genetics **93**: 189-200.

Burnouf, T. and Bouriquet, R.1980. Glutenin subunits of genetically related European hexaploid wheat cultivars : their relation to bread making quality. Theor. Appl. Genet. **58**: 107-111.

Feldman, M. and Sears, E.R. 1981. The wild gene resources of wheat. Scientific Amer. **244**: 102-112.

Galili, G. and Feldman, M.1985.Genetic control of endosperm proteins in wheat. Theor. Appl. Genet. **69**; 583-589.

Law, C.N. and Payne P.I. 1983a. Genetical aspects of breeding for improved grain protein content and type wheat. J. Cereal Sci. **1**: 79-83.

Law, C. N. 1983b. The high-molecular weight subunits of glutenins: Classical genetics, molecular genetics and the relationship to bread making quality. Proc. 6th Int. Wheat Genet. Symp., Kyoto, Japan, pp. 827-834.

Lawrance, G. J., and Shepherd, K.W. 1980. Variation in glutenin protein subunits of wheat. Aust. J. Biol. **33**: 221-223.

Lawrance, G. J. and Shepherd, K. W. 1981a. Chromosomal location of genes controlling seed proteins in species related to wheat. Theor. Appl. Genet. **59**: 25-31.

Lawrance, G.J. and Shepherd, G. J. 1981b. Inheritance of glutenin protein subunits of wheat. Theor. Appl. Genet. **60**: 333-337.

Laemmlil, U.K.1970. Cleavage of structural proteins during the assembly of the head of bactriophage T4. Nature **227**:680-685.

Moonen, J.H.E., Scheepstra, A. and Graveland, A. 1982. Use of the SDS-sedimentation test and SDS-polyacrylamide gel electrophoresis for screening breeder's samples of wheat for bread making quality. Euphytica **31**: 677-690.

Nevo, E. and Payne. P. I. 1987. Wheat storage protein: Diversity of HMW- glutenin subunits in wild emmer from Israel.Theor. Appl. Genet. **74**: 827-836.

Payne, P. I. and Corfield, K.G. 1979a. Subunit composition of wheat glutenin protenins, isolated by gel filtration in a dissociating medium.Planta. **145**: 83-88.

Payne, P. I., Corfield, K. G. and Black, J. A. 1979b. Identification of a high-molecular weight subunit of glutenin whose presence correlates with the bread making quality in wheat of related pedigree. Theor. Appl. Genet. **55**: 153-159.

Payne, P. I., Law, C. N. and Mudd, E. E. 1980. Control by homoeologous group 1 chromosomes of the high-molecular weight subunits of glutenin, a major protein of the wheat endosperm. Theor. Appl. Genet. **58**: 113-120.

Payne, P .I., Corfield, K.G., Holt, L. M. and Black, J. A. 1981a. Correlations between the inheritance of certain high-molecular weight subunits of glutenins and bread making quality in progenies of six crosses of bread wheat. J. Sci. Fd. Agric. **32**: 51-60.

Payne, P.I. Holt, L.M. and Law, C.N. 1981b. Structural and genetical studies on the high-molecular weight subunit of wheat glutenin. Theor. Appl. Genet. **60**: 229-236.

Payne, P. I., Holt, L. M., Lawrance, G. J. and Law, C. N. 1982a. The genetics of gliadins and glutenins, the major storage proteins of wheat endosperm. Qualitas Plant. Pl. Fds. Hum. Nutr. **31**: 229-241.

Payne, P. I., Holt, L. M., Worland, A. J. and Law, C. N. 1982b. Structural and genetical studies on the high-molecular weight subunits of wheat glutenins. 3. Telocentric mapping of the subunit genes on the long arms of the homoeologous group 1 chromosomes. Theor. Appl. Genet. **63**: 129-138.

Payne, P. I., Holt, L. M. and Lawrance, G. J. 1983a. Detection of a novel high-molecular weight subunit of glutenin in some Japanese hexaploid wheats. J. Cereal Sci. 1:3-8.

Payne, P.I. and Lawrance, G. J. 1983 b. Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1 which code for high- molecular weight subunits of glutenin in hexaploid wheat. Cereal Res. Comm. **11**: 29-35.

Payne, P. I., Holt, L.M., Jackson, E.A. and Law, C.N.Low 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Phil.Trans.R.Soc.Lond. B **304**:359-371.

Shewry,R. R., Tatham, A. S., Forde, J., Kreis,M. and Miflin, B. J.1986. The classification and nomenclature of wheat gluten proteins: a reassessment. J. Cer. Sci. **4**: 97-106.

Shewry, R. R. and Tatham, A. S. 1989. The high-molecular weight subunits of wheat, barley and rye : Genetics, molecular biology, chemistry and role in wheat gluten structure and functionality. Vol 6, pp. 163-219. In Oxford Survey of Plant Molecular and Cell Biology.

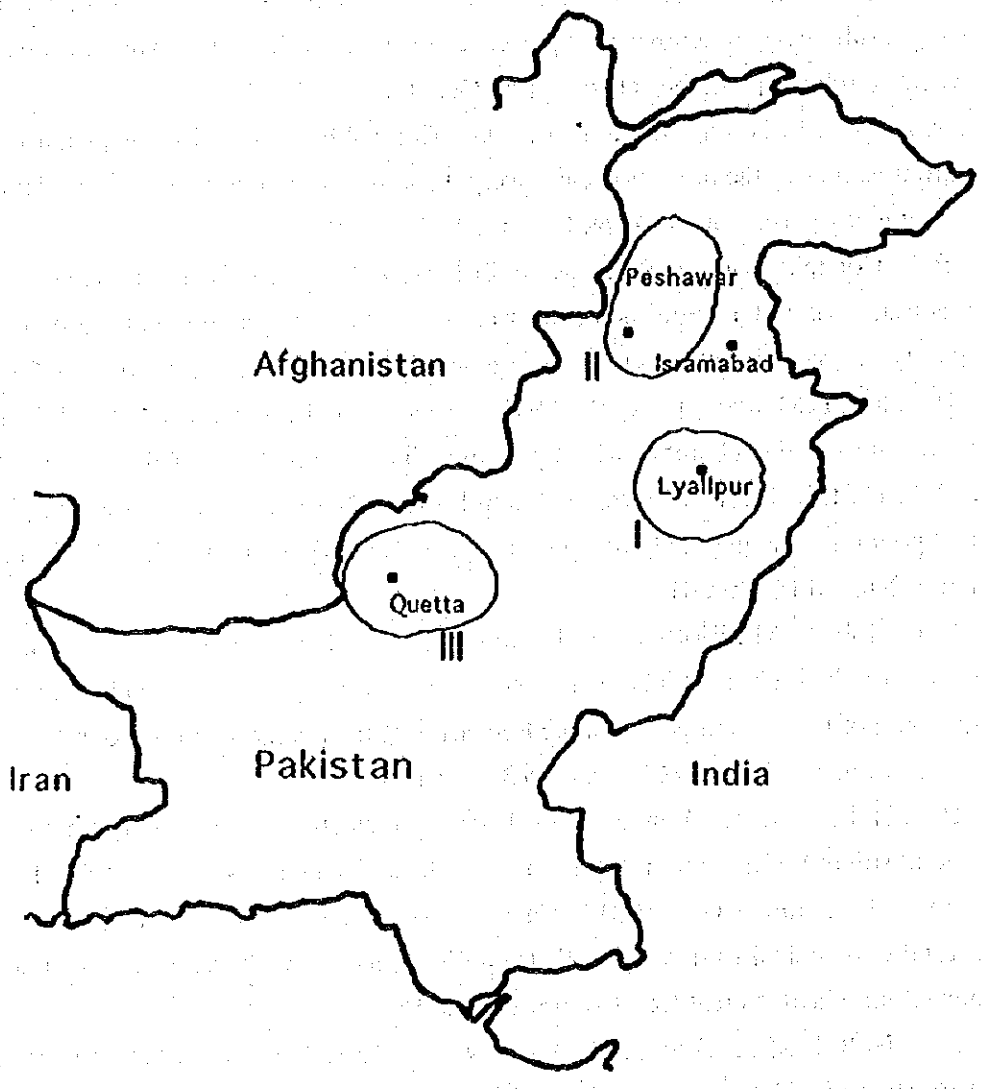

Fig .1 Three locations of Pakistani wheat lines used in the experiment

I Punjab Province
II N. W. F. P.
III Baluchistan Province

Table 1 Classification of Pakistani lines by the combination of Glu -A1, Glu -B1 and Glu -D1

Line Group	Number	Glu-A1	Glu-B1	Glu-D1
A. Pakistani land race WB 6, WB 8, WB 20, WB 27, WB 44, WB 138, WB 357, WB 612, WB 17-3, WB 44-1, WB 138-1, QT 105, QT 105-1, QT 106, QT 108, (49P 70-2), (49P 70-5), (49P 70-7), (49P 70-16), (49P 70-20), (49P 74-4),(49P 75-5), (49P 77-11), (49P 78-5), (49P 79-13), (49P 80-1),(49P 81-7), (49P 81-8), (49P 87-7), Localwhite, Localmix.	31	Null	7+8	2+12
B. Pakistani land race (49P 70-17), (49P 70-34),(49P 71-7), (49P 71-8), (49P 71-9), (49P 72-30), (49P 81-12), (49P 82-3), (49P 82-20), (49P 85-1).	10	1	7+8	10
C. C 299, B.D 63, Tamab 18,Tarnab 75, C 271 Son 64, Dirk, Black Awn Khushal.	7	1	7	5+10
D. Pakistani land race (49P 34)-a,WB 17-2, WB 597, C228, Tamab 107, QT 107.	6	2*	17+18	2+12
E. Pu Thway, NPS 76 Olesen/5661, Pakistani land race (49P 73-7), (49P 76-6), (49P 84-6), (49P 86-6).	6	2*	7+8	2+12
F. Pakistani land race (49P 34)-b, WB 17, WB 17-1,WB 357, WB 565, M 15.	6	Null	17+18	2+12
G. Lyallpur 73, Sandol, SA. 42, Azteca 66.	4	1	17+18	5+10
H. Chenab 70, Tarnab 87/73, SA.42.	3	1	17+18	2+12
I. Triple Dwarf, Mida/N.Th.-K 117 A /Ind. 38 Son.64, Pari 73.	3	Null	17+18	5+10
J. Barani 70, Pakistani land race WB 189.	2	Null	20	2+12
K. Ksh S-M. Pak 66, Pakistani land race (49P 34)-c.	2	2*	20	2+12
L.CNO S/HDS 32-5-5-5-OY,Pakistani land race (49P 71-10).	2	1	7+8	5+10
M. Sonalika.	1	2*	7+9	2+12
N. Khushal 69-a	1	1	20	2+12
O. Khushal 69-b	1	1	20	5+10
P. Tarnab 87.	1	1	7	2+12
Q. Son 64/C 271/235 S 4.	1	2*	20	5+10
R. Pakistani land race(49P34)-d	1	Null	20	12
Total:	88			

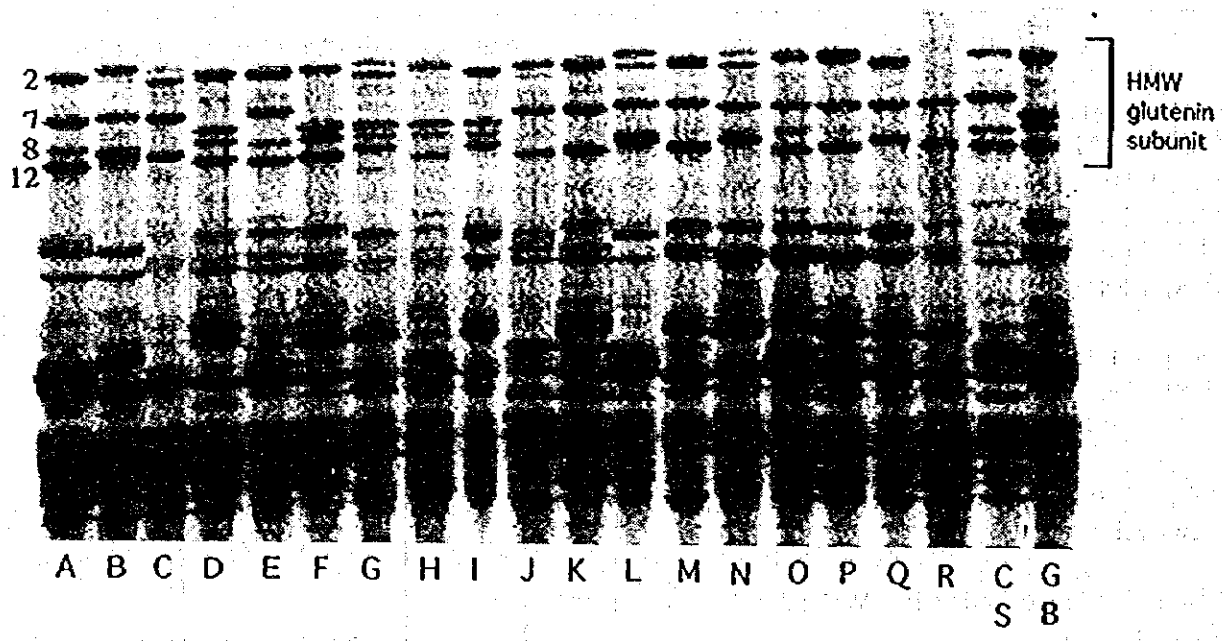

Fig. 2. SDS-PAGE patterns of HMW subunits of glutenin from 18 groups of Pakistani wheat lines and two standard cultivars. Cultivars are follows: A,Pakistani landrace(49P79-13);B,Pakistani landrace(49P82-3);C,Dirk;D,Pakistani landrace(49P34)-a;E,NPS76 Olesen/5661;F,Pakistani landrace(49P34)-b; G,Azteca 66;H,Chenab 70;I,Triple dwarf;J,Pakistani landrace WB189; K,Pakistani landrace(49P34)-c;L,Pakistani landrace(49P71-10); M,Sonalika; N,Khushal 69-a;O,Khushal 69-b;P,Tanab 87;Q,Son 64/C271/ 235 S4;R,Pakistani landrace(49P34)-d;CS,Chinese spring;GB,Gabo

Table 2 The genotypes of standard cultivars compared with Pakistani lines

Cultivar's name	Origin	Glu-A1	Glu-B1	Glu-D1
Mexipak	CIMMYT	2*	17+18	2+12
Mexipak 65	CIMMYT	2*	17+18	2+12
Sonora 64	CIMMYT	1	17+18	5+10
Mexico 120	CIMMYT	Null	7+8	2+12
Blue Silver	CIMMYT	2*	7+9	2+12
Chinese Spring	China	Null	7+8	2+12
Gabo	Australia	2*	17+18	2+12
Norin 10	Japan	1	7+8	4+12
Eshimashinriki	Japan	2*	7+8	2+12
Shinchunaga	Japan	Null	7+8	2.2+12
Norin 61	Japan	2*	7+8	2.2+12

Table 3 The gene frequencies in Glu-A1, Glu-B1 and Glu-D1 loci for Pakistani landraces, breeding lines and CIMMYT breeding lines compared with Japanese breeding lines

	Glu-A1		Glu-B1		Glu-D1	
Group	subunits	No. of lines (%)	subunits	No. of lines (%)	subunits	No. of lines (%)
Pakistani landraces (58)	1	12 (21%)	7	0 (0%)	**2+12**	**46 (79%)**
	2*	9 (15%)	**7+8**	**46 (79%)**	4+12	0 (0%)
	Null	**37 (64%)**	7+9	0 (0%)	5+10	1 (2%)
			20	3 (5%)	10	10 (17%)
			14+15	0 (0%)	12	1 (2%)
			17+18	9 (16%)		
Pakistani breeding lines (30)	**1**	**18 (60%)**	7	8 (27%)	**2+12**	**13 (43%)**
	2*	7(23%)	7+8	3 (10%)	4+12	0 (0%)
	Null	5(17%)	7+9	1 (3%)	**5+10**	**17 (57%)**
			20	5 (17%)	10	0 (0%)
			14+15	0 (0%)	12	0 (0%)
			17+18	**13 (43%)**		
CIMMYT breeding lines (5)	1	1 (20%)	7	0 (0%)	2+12	4 (80%)
	2*	3 (60%)	7+8	1 (20%)	4+12	0 (0%)
	Null	1 (20%)	7+9	1 (20%)	5+10	1 (20%)
			20	0 (0%)	10	0 (0%)
			14+15	0 (0%)	12	0 (0%)
Total:93			17+18	3 (60%)		
Japanese breeding lines (21)	1	5 (24%)	7	0 (0%)	**2+12**	**7 (33%)**
	2*	6 (28%)	**7+8**	**12 (57%)**	**2.2+12**	**9 (43%)**
	Null	**10 (48%)**	7+9	4 (19%)	4+12	5 (24%)
			20	0 (0%)	5+10	0 (0%)
			14+15	2 (10%)	10	0 (0%)
			17+18	3(14%)	12	0 (0%)

Appendix

Sample preparation

Two grams of each wheat seed sample were milled. These flour samples were sieved by a 100-mesh sieve and stored in small glass bottles, separately. After this out of each glass bottle 40 mg of flour of each sample was taken in a eppendorf microcentrifugation tube and mixed with 1 ml of SDS-buffer solution and completely solubilized and stirred on automatic operating vortex mixture, till the whole flour was completely solubilize. To solubilize the protein a sample was shaked in the automatic shaker for 120 minutes. After this these samples were boiled in boiling water for 3 minutes, then rotated/centrifuged for 13 minutes at 12,000 rpm, and the supernatant was used for SDS-PAGE technique.

Gel preparation

The specified glass gel plates having 1mm thick shield were connected to each other with the help of 4 clips. 7.5 % polyacrylamide solution was prepared by using 29.2 grams of acrylamide mixed with 0.8 grams of methylene BIS acrylamide, and water was added to it upto the volume of 100 ml. This solution was kept in cool and dark conditions. Stacking gel solution was prepared by using 3.02 grams of 2-Amino-2 hydroxymethyl-1,3-propanediol [Tris (hydroxymethyl) aminomethane] and sodium dodecyl sulphate (SDS) 0.2 grams and added 80 ml water, and the solution was made upto pH 6.8 by addition of concentrated HCl and the final volume raised upto 100 ml. Separation gel buffer was prepared by taking 18.165 grams Tris mixed with 0.4 grams SDS, adding 80 ml of water and using conc. HCl for pH up to 8.8 and final volume upto 100 ml by adding more water. Electrophoresis buffer was prepared by mixing 3.025 grams Tris with 1.0 grams SDS and 14.4 grams glycine and 1000 ml water.

For preparation of separation gel, 8.0 ml of 7.50 % polyacrylamide solution was taken in beaker, added 11.0 ml of separation gel buffer and 13.0 ml of distilled water. Then 240 µl of 10% ammonium persulphate was added and stirred very thoroughly. 40 µl of TEMED was mixed and immediately solution was poured in the gel glass plates, leaving 3.5 cm space on the upper portion of the plates. The surface of the gel was covered by adding 1ml distilled water for getting a fine and sharp surface. Stacking gel was prepared by mixing 2 ml of 7.50% polyacrylamide solution with 6 ml of stacking gel buffer, 4 ml of water and 60 µl of 10% ammonium persulphate, stirred on automatic stirrer for one or two minutes and 40 µl of

TEMED was added to this solution and after removing the water from the separation gel this solution was poured on its surface rapidly and 20 wells plastic comb was inserted in the plates on the upper side.

The already prepared electrophoresis buffer was poured in the electrophoresis apparatus and within half to one hour the plastic comb and the four clips were removed from the original glass plates, and these plates were fitted tightly in the electrophoresis apparatus having electrophoresis buffer. More buffer was added in the apparatus so that one of the gel glass plates was completely dipped in it and the stacking gel was covered by the buffer. Just before the electrophoresis, 5 µl of sample extract was loaded into each well with a micro syringe. At a set time 20 different samples were loaded in 20 wells of a single gel, being one sample extract in each well.

Electrophoresis

The total seed protein was analyzed with the slab type SDS-PAGE following the method of Laemmli (1970) using 10 % polyacrylamide gel. The black and red terminals were connected with the power apparatus. The electrophoresis was performed at a current of 16 mA for about eight hours. After that the gels were removed from the glass plates very carefully and all the gels were stained with Coomassie brilliant blue R 250 for about 20-30 minutes and then destained in a 25% methanol-7.5% acetic acid solution till the color of the back ground disappeared and the protein bands became clearly visible. After destaining, the gels were kept in the Gel Drying Processor and were dried for 80 minutes.

Separation Gel Buffer (1.5 M Tris-HCl, pH 8.8/ 0.4 % SDS)

Tris-HCl	18.165 g
SDS	0.4 g
Water	80 ml
↓	
Addition of conc. HCl until pH 8.8	
↓	
Addition of much water upto final volume	100 ml .

Stacking Gel Buffer (0.25 M Tris-HCl , pH 6.8/ 0.2 % SDS)

Tris	3.025 g
SDS	0.2 g
Water	80 ml
↓	
Addition of 1 N HCl until	pH 6.8
↓	
Addition of much water upto final volume	100 ml.

Electrophoresis Buffer (Glycine 192 mM, 25 mM Tris-HCl, 0.1 % SDS pH 8.5)

Tris-HCl	3.025 g
SDS	1.0 g
Glycine	14.4 g
Water	1000 ml

Staining Solution (0.1 % coomassie brilliant blue R-250, 50 % Methanol, 10 % Acetic acid)

Coomassie brilliant blue R-250	1.0 g
Methanol	500 ml
Acetic acid	100 ml
↓	
Final volume upto by addition of dist. water	1000 ml

Destaining Solution (25 % Methanol , 7.5 % Acetic acid)

Methanol	250 ml
Acetic acid	75 ml
↓	
Adding dist. water upto final volume	1000 ml

Preparation of Gels

Separation gel prepared by

7.5% polyacrylamide solution	8 ml
Separation gel buffer	11 ml
Distilled water	13 ml
10 % ammonium persulphate (APS)	240 µl

stirred

↓

TEMED	40 µl

immediately poured into glass plates and covered the upper surface by pouring water 1 ml.

Stacking gel prepared by

7.5 % polyacrylamide solution	2 ml
Stacking gel buffer	6 ml
Distilled water	4 ml
10 % APS	60 µl

↓

stirred for 2 minutes.

↓

TEMED	40 µl

immediately poured on the surface of separation gel after removing water and comb was inserted in it.

Sample Preparation

Seeds milled by a 100-mesh sieve, flour filtrated	2 g

↓

Flour in eppendorf microcentrifuge tube	40 mg

↓

SDS buffer	1 ml

↓

Stirring on vortex mixture and solubilized

↓

Shaking for 2 hours

↓

Boiling samples for 3 minutes

↓

Centrifugation at 12,000 rpm for 13 minutes and suspended

↓

5 µl sample for SDS-PAGE

7. CHILE

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NATIONAL INSTITUTE OF
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JAPAN INTERNATIONAL COOPERATION AGENCY (JICA)

TECHNICAL REPORT

PLANT GENETIC RESOURCES COURSE

SEED DORMANCY AND LONGEVITY IN INDICA AND JAPONICA RICE VARIETIES

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JAPAN, 1995

SUMMARY

Seed dormancy and longevity were investigated in 26 Indica and 69 Japonica rice varieties. Indica group showed longer seed longevity than Japonica under accelerated aging conditions (40°C, 90% RH, 10 days). On the other hand, Indica group showed lower degree of seed dormancy than Japonica, in view of seed germination comparison between Indica (76%) and Japonica (44%) in control condition and brown rice treatment (glumes were removed).

The correlation between the degree of seed dormancy and seed longevity was not found in all materials which include Indica and Japonica varieties and samples of different transplanting times.

INTRODUCTION

Rice (*Oriza sativa* L.) is the more important cereal crop being grown in the world (144.641 million ha), and a 91% of the world rice production and 91% of global rice consumption is concentrated in Asia (IRRI Rice Facts 1988, in Bajaj 1991). Rice also, is one of the more ancient crop; there exist evidence that was cultivated in southern China at least 7000 years ago (Bajaj 1991).

The long history of use for human being together with its cultivation in a large variation of environmental had produced a great rice genetic diversity that show a large number of varieties and landraces. They also are very important genetic resources, because they contain resistant genes to diseases and important agronomic characteristics. A large number of accessions with wide genetic diversity preserved at the rice gene banks in the world (NIAR-Japan, 23000; IRRI-Philippines, 8000; National Gene Bank-China, 50000). *Oriza sativa* L. which occupy a large part of rice materials preserved in the rice gene banks are classified in two varietal groups namely Indica and Japonica (Chandler 1979, Vaughan 1994). The two groups differ at morphological, biochemistry, agronomic, physiological and genetic level (Table 1).

If there are the difference of characters regarding seed between Indica and japonica groups, and within each groups, they are profitable for activities related to seed management in storage.

Oka & Tsai (1955), found that Indica varieties have a tendency of longer seed longevity in storage and to show less level of dormancy compared with Japonicas. Roberts (1963) view that when seed is stored under identical condition, the seed dormancy period may differs markedly between varieties while the variability remains the same. Sato (1991) report that there is the difference on seed longevity between upland (rain water supply culture) and lowland (sumerged soil culture). They are classified according to the difference of the water stress resistance.

The objectives of this work are to evaluate the difference of: i) Seed dormancy and longevity between Japonica and Indica rice varieties, and the relationship between seed dormancy and longevity and, ii) seed longevity between upland and lowland rice varieties.

MATERIALS AND METHODS

The most important factors that influence the seed survival during storage are temperature and seed moisture content (Harrington 1973). This means that high temperature and high humidity bring rapid deterioration of seeds. Delouche & Baskin (1973) say that accelerated or artificial aging of seed lots over several days of exposure to 40°C and saturity humidity has been recognized as a good predictor od storability. Accelerated aging has subsequently been recognized as a useful vigor test for some species (Association of Official Seed Analysist Test Committee 1983).

a) Seed longevity and dormancy in Indica and Japonica varieties.

A preliminary aging test was carried out using 6 Indica and 6 Japonica varieties to define the optimum period of aging treatment to detect clear differences in seed viability between two groups. In this test, seed plots (50 seed each one) were exposed to 5, 10, 15, 20, 25 and 30 days under 40°C and 90% RH. Germination percentage was counted at 10 days after sowing under 30°C of daytime (8 hours) and 20°C of night time.

True seed viability is evaluated by taking off the dormancy. There are several methods to break the seed dormancy. The high temperature treatment and the hull removal are effective methods to break dormancy. Breaking dormancy test was carried out using eight varieties including both strong and weak dormancy. 25 seeds each one was put in 3, 6, 9 and 12 days under 50°C, and germination rate was investigated with the similar method to preliminary aging test. In this test, since the seed dormancy of two varieties was not broken with 12 days treatment, which is the longest (Fig. 1a,b), I decided to use brown rice materials as the better method to evaluate the true seed viability.

The difference between Indica and Japonica rice on longevity and seed dormancy was investigated using samples (50 seed each one) of 25 Indica and 69 Japonica varieties. This test is consist of three treatments such as bellow:

i) Control: No aging treatment and unhulled rice. This plot indicate the degree of dormancy.

ii) Brown rice: Seed glumnes were removed with the purpose of broken dormancy. This plot indicate the true seed viability.

iii) Aging: Unhulled rice samples incubated for 10 days in aging chamber under 40°C and 90% Relative Humidity. Seed longevity was evaluated with the germination percentage of this plot.

In the Brown rice and Aging plot, the seed germination was investigated according to International Seed Testing Association Rules (ISTA 1985). The seed germination in control plot was continuously evaluated at every 5 days from 10 to 30 days after sowing.

All samples used in this test were harvested 1994 and stored directly in the medium-term storage room (-1°C, 30% relative humidity) of the MAFF Gene Bank of NIAR.

b) Seed longevity in F_2 crossing population between upland and lowland varieties.

The difference of seed longevity between lowland and upland rice was investigated using F_2 populations between 6 reciprocal crosses (only one side) of the following parental varieties: Sasanishiki, Mangetsumochi (lowland) and Nourin 21, Tsukubahatamochi (upland). The plot numbers of crosses is shown in Table 1. The aging condition and germination testing method was similar to before test.

All samples used in this test, were harvested in 1994, and after drying to 35°C in 4 days, maintained for 1 month in working room (25°C) and later preserved in the medium-term storage room (-1°C, 30% relative humidity), of MAFF Gene Bank of NIAR.

RESULTS AND DISCUSSION

In the preliminary aging test, it is shown that the aging treatment of 40°C and 90% relative humidity decrease seed germination percentage, and the treatment days bigger difference among varieties are 10 and 15 days (Fig. 2a,b). Therefore, I selected the 10 days aging treatment which is available to get the result more earlier for the main aging test.

The mean germination percentage of the control, the brown rice and the aging plot in the main test are shown in Fig. 3. The mean germination percentage in the brown rice plot which show the true seed viability is almost same between Indica and Japonica group. In the other hand, there are big differences between both group in the control and aging plot. The mean germination percentage of Indica group (69%) in the aging plot was significantly larger ($t = 9.7$; $P<0.001$) than Japonica (19%). The germination percentage under the aging treatment agree very well with the seed longevity. These results are similar to that found by Oka & Tsai (1955) and Ellis et al. (1992, 1993) and different from that obtained by Roberts (1963). It is possible to consider that the different results in these reports are assigned to samples size, because in the two studies already mentioned the

number of varieties analyzed was very small (2 Indica and 3 Japonica, Roberts 1963; 3 Indica and 3 Japonica varieties, Oka & Tsai 1955; 2/1 and 1/2 in Ellis et al. 1992, 1993, respectively). At is possible to say that the seed longevity of Indica varieties are generally longer compared with Japonica varieties. But we have to pay attention that there are big variation of seed longevity within both group (Fig. 4).

In the mean germination percentage at 10 days in the control plot, Indica group show the higher value (76%) than Japonica (44%) (Fig. 3), and the difference between two group is significant statistically ($t = 4.45$; $P<0.001$). Since the germination percentage of the both groups in the brown rice plot are almost same (Fig. 3), it is concluded that Indica rice has lower degree of seed dormancy that Japonica rice.

However, the data used in this analysis concern to 10 days after sowing date. At end of the experiment (day 30) the germination percent were very similar (Indica: 84 ± 21; Japonica: 87 ± 17). A frequency distribution analysis of germination percents (Fig. 5), show that in the 10 day a high percent of Japonica varieties had low seed germination (Fig. 5a). In 30 day the frequency distribution between both varietal group was very similar (Fig. 5b). This suggest that: First, the degree of dormancy is a character highly variable within both rice varietal groups. Second, the Japonica varieties had stronger dormancy than Indica varieties.

The correlation between the degree of seed dormancy and seed longevity was not found in all materials which include Indica and Japonica varieties and samples of different transplanting times

(Fig. 6). Since there are one different report on the relationship of the seed dormancy between seed longevity (Ota & Takemura 1970), it is needs the further discussions on this problems.

Contrary to the expectancy (see: Sato 1994), no differents in seed longevity among lowland and upland rice varieties was found. This is showed comparing the seed germination between the four varieties analyzed (Fig. 7a). Moreover, the seed germination in crosses was very similar to parent varieties (Fig. 7b).

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BIBLIOGRAPHY

Association of Official Seed Analyst, Seed Vigor Test Committee (1983) Seed Vogot Testing handbook. N.p.

Bajaj YPS (1991) Biotechnology in Rice Improvement. In: Rice: 4-18. Bajaj YPS (Ed). Biotechnology in Agriculture and Forestry Vol. 14. Springer-Verlag Berlin, Heidelberg.

Chandler R (1979) Rice in the Tropics: A Guide to the Development of National Programs. Westview Press, Boulder, Colorado.

Delouche JC & CC Baskin (1973) Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci. Technol. 1:427-452.

Ellis RH, TD Hong and EH Roberts (1992) The low-moisture-content limit to the negative logarithmic relation between seed longevity and moisture content in three subspecies of rice. Annals of Botany 69:53-58.

Ellis RH, TD Hong & MT Janckson (1993) Seed production environment, time of harvest, and the potential longevity of seed of three cultivars of Rice (*Oriza sativa* L.). Annals of Botany 72:583-590.

Harrington (1973) Problems of Seed Storage. In: Seed Ecology: 251-263. Heydecker W (Ed.). The Pennsylvania State University Press, London.

International Seed Testing Association, ISTA (1985) International Rules for Seed Testing. Seed Science and Technology 13: 299-355.

Murayama S, M Kabaki & K Tajima (1985) Water consumptions in Japonica and Indica rice Varieties. Japan Jour. Crop. Sci. 54: 32-38.

Otto E & A Osada (1984) Different response between Indica and Japonica rice varieties to Nitrogen fertilizer as expressed by physiological and morphological characters. Japan J. trop. Agr. 28: 13-24.

Oka HI & KH Tsai (1955) phylogenetic differentiation of cultivated rice. 10. Dormancy and longevity of rice seed with regard to their variation among varieties. Jpn. J. Breed. 5:22-26. (Japanese; English Summary).

Oka HI (1988) Indica-Japonica Differentiation of Rice Cultivars. In: Origin of Rice Cultivated Rice: 141-179. Japan Scientific Societies Press, Tokyo.

Osada A, Y Ishizaki & S Suzuki (1983) difference in the number of days for ripening of grains between Japonica and Indica rice. Japan J. Trop. Agr. 27:59-66.

Ota Y & Y Takemura (1970) Rice storage and seed dormancy. Journal of Agricultural Science 25:218-222. (Japanese).

Roberts EH (1963) An Investigation of inter-varietal differences in dormancy and viability of rice seed. Annals of Botany 27:365-369.

Summerfield RJ, Collinson ST, RE Ellis, EH Roberts & FWT Penning de Vries (1992) Photothermal responses of flowering in rice (*Oriza sativa*). Annals of Botany 69:101-112.

Sato M (1991) Studies on seed longevity of crops in long-term storage. Report of Agricultural & Biological Resources Institute 6: 98-110. (Japanese; English Summary)

Samarray SM (1969) Studies on varietal difference in productivity on related characters between Japonica and Indica rices their genetic behaviour. I. Plant characters related to varietal differences in productivity between japonica and indica varieties if rice plant. Proc. Crop Sci. Soc. Japan. 38:627-646.

Taira H & WL Chang (1986) Lipid contend and fatty acid composition of Indica and Japonica Types of Nonglutinous Brown rice. J. Agric. food Chem. 34:542-545.

Vaughan DA (1994) The Wild Relatives of Rice. A Genetic Resources Handbook. IRRI, Manila, Philippines.

Weng JH & CY Chen (1987) Differences between Indica and Japonica rice varieties in CO_2 exchange rates in response to leaf Nitrogen and temperature. Photosynthesis Research 14:171-178.

Table 1. Differences between the Indica and Japonica rice varieties on characters of agronomic importance.

CHARACTER	INDICA	JAPONICA	References
1. Biochemistry			
Lipid content	Low	High	Taira & Chang (1986)
Fatty Acids	High	Low	Taira & Chang (1986)
Amylase content	High	Low	Horiuchi & Tani (1966)[1]
2. Agronomic			
Cold resistance of Seedling	Low	High	Oka (1958)[2]
Drought resistance Seedling	High	Low	Oka (1958)[2]
Lodging resistence	Low	High	Matsuo (1952)[2]
Days of ripening grains	Least	Greater	Osada et al. (1983)
Water consumptions	High	Low	Maruyama et al. (1985)
Weight of dry matter in ripering	Low	High	Samarrai 1969
Panicle yield	Low	High	Samarrai 1969
Starch in leaves	Low	High	Samarrai 1969
3. Physiology			
T° and Leaf N_2 reponse of:			
Photosyntetic rate	High	Low	Weng & Chen (1987)
Respiratory rate	Low	High	Weng & Chen (1987)
Nitrogen response of:			
Protein conten	Low	High	Otto & Osada (1984)
Photosynthetic activity	Low	High	Otto & Osada (1984)
Sensivility to temperature	Less	More	Summerfield et al (1992)
Sensivility to photoperiod	More	Less	Summerfield et al (1992)
4. Genetic			
Diversity of isozime alleles	High	Low	Oka (1988)

1 In Taira & Chang (1986)
2 In Oka (1988)

Table 2. Cross combination and number of plots among lowland and upland varieties. The total plots for each one parental varieties was 30.

No.	Cross combination	No. of Plots
1	Sasanishiki/Mangetsumochi	148
2	Sasanishiki/Nourin 21	145
3	Sasanishiki/tsukubahatamochi	148
4	Mangetsumochi/Nourin 21	152
5	Mangetsumochi/Tsukubahatamochi	154
6	Nourin 21/Tsukubahatamochi	146

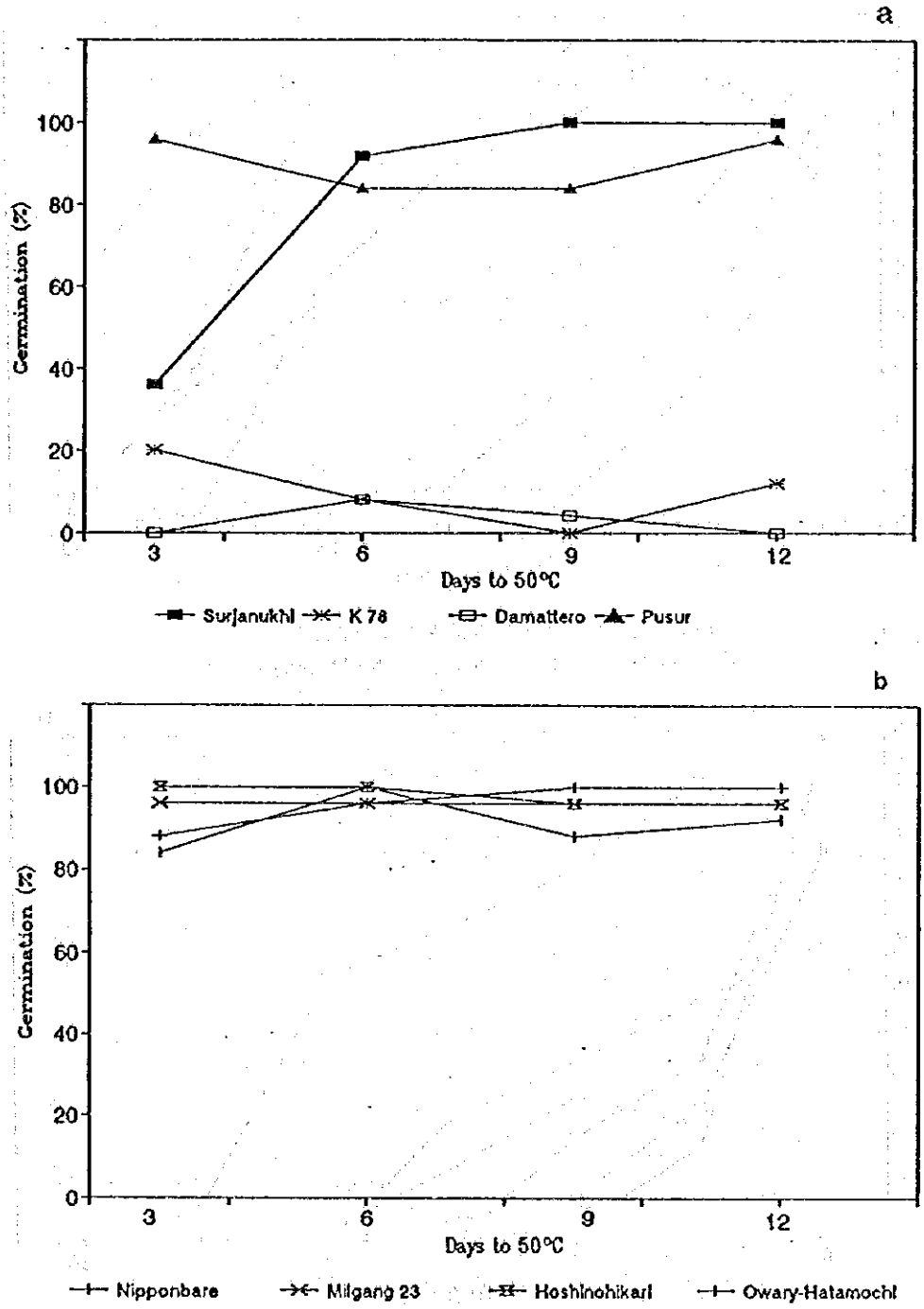

Fig. 1. Effect of breaken dormancy days to 50°C in the germination of indica and japonica varieties. (a) Strong dormancy varieties, (b) Weak dormancy varieties.

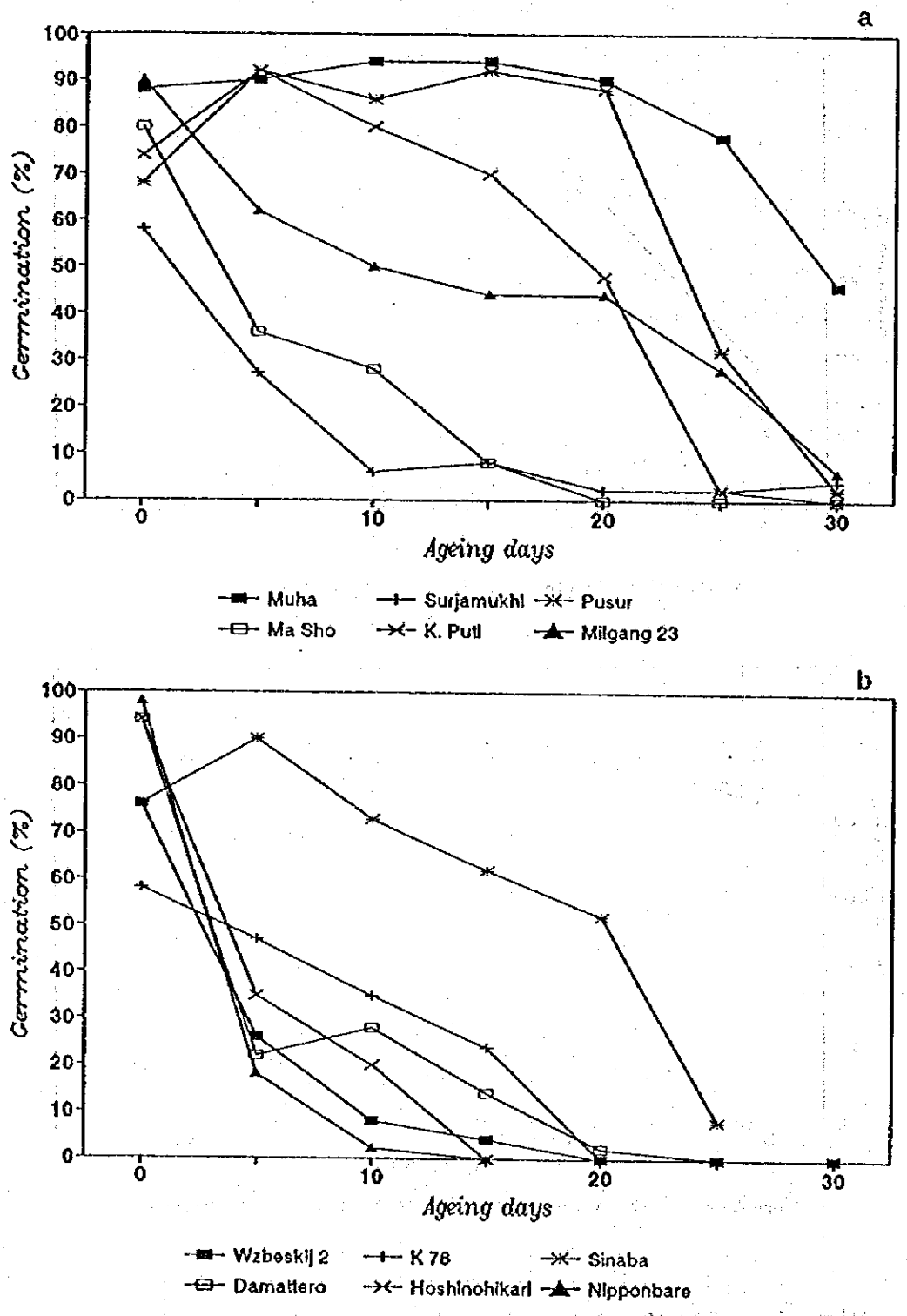

Fig. 2. Effect of number of days under aging condition (40°C, 90% RH) in the seed germination of indica (a) and japonica (b) varieties.

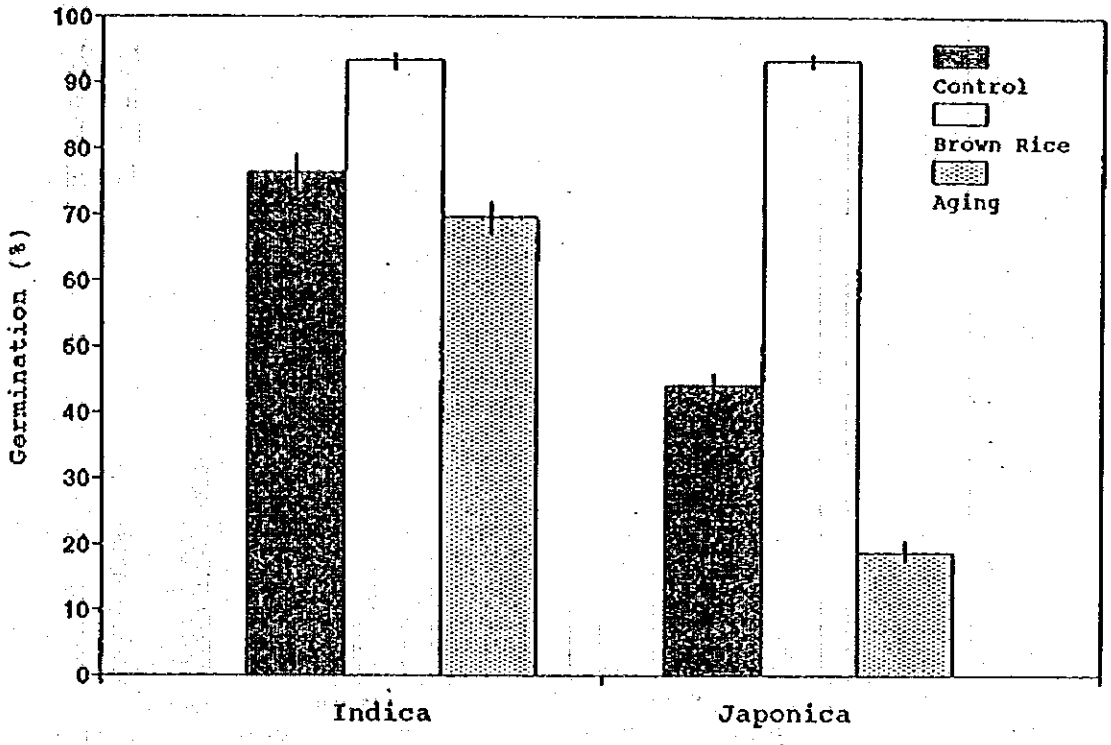

Fig. 3. Germination percentage of control plot, brawn rice and aging treatment in indica and Japonica varieties. Average ± EE.

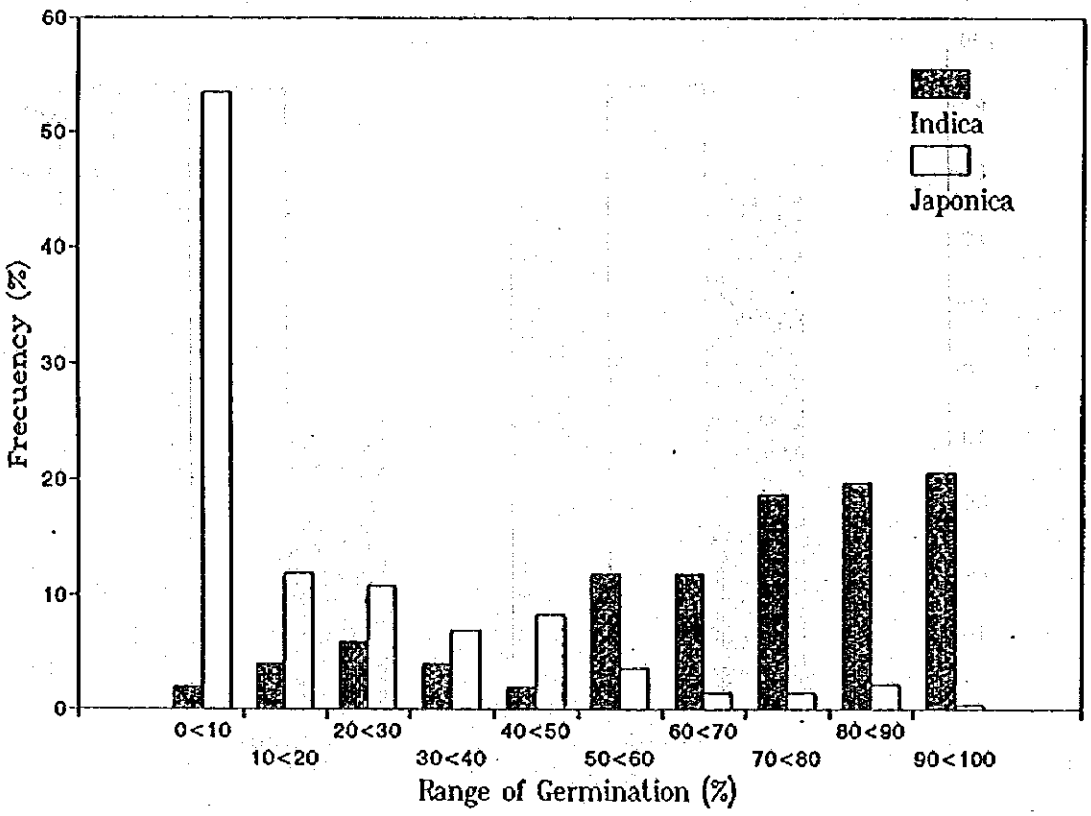

Fig. 4. Frequency distribution of germination percentage of indica and japonica varieties under aging condition (40°C, 90% RH, 10 days).

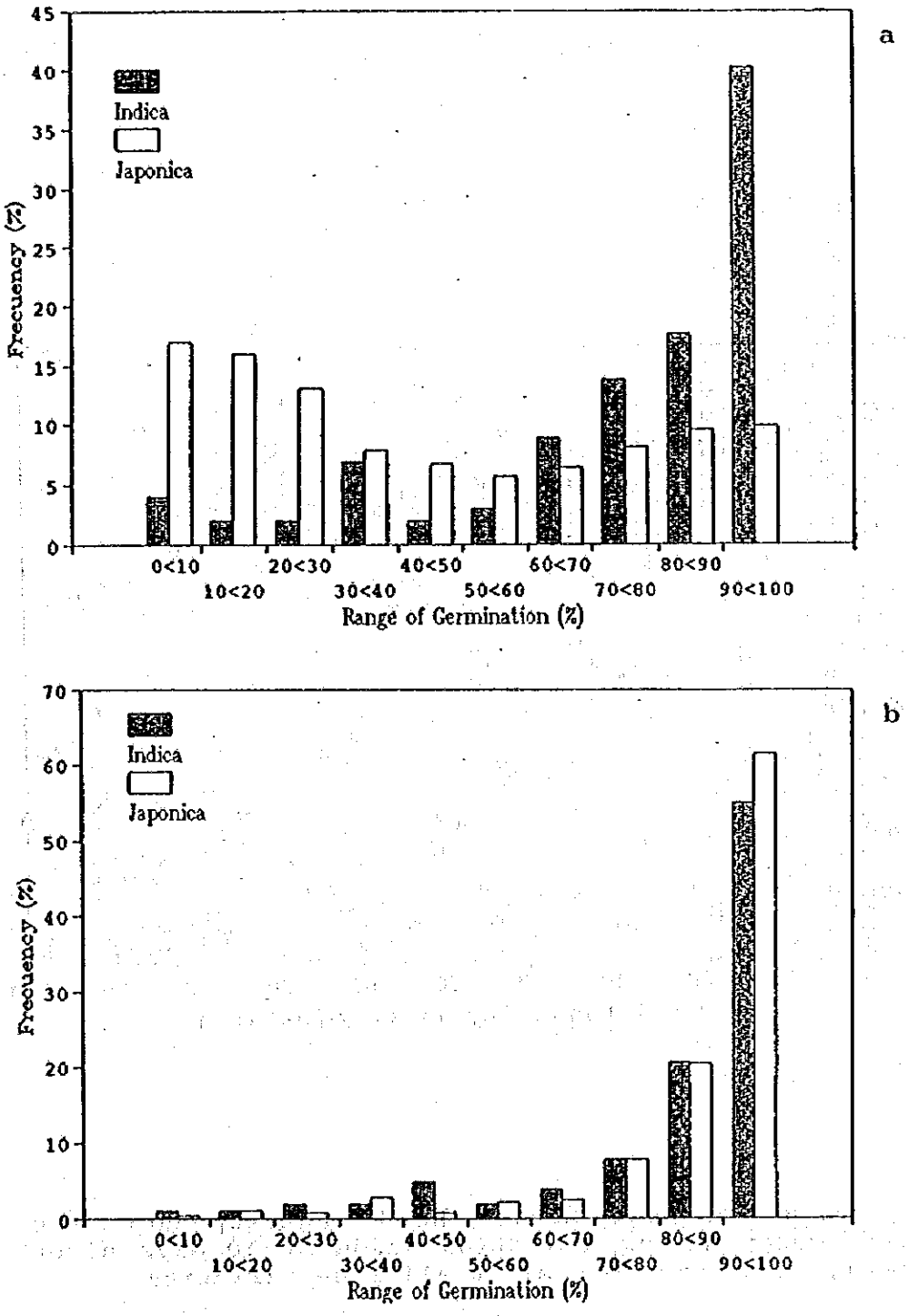

Fig. 5. Frequency distribution of germination percentage of indica and japonica varieties in control plot. (a) 10 and b) 30 days after sowing.

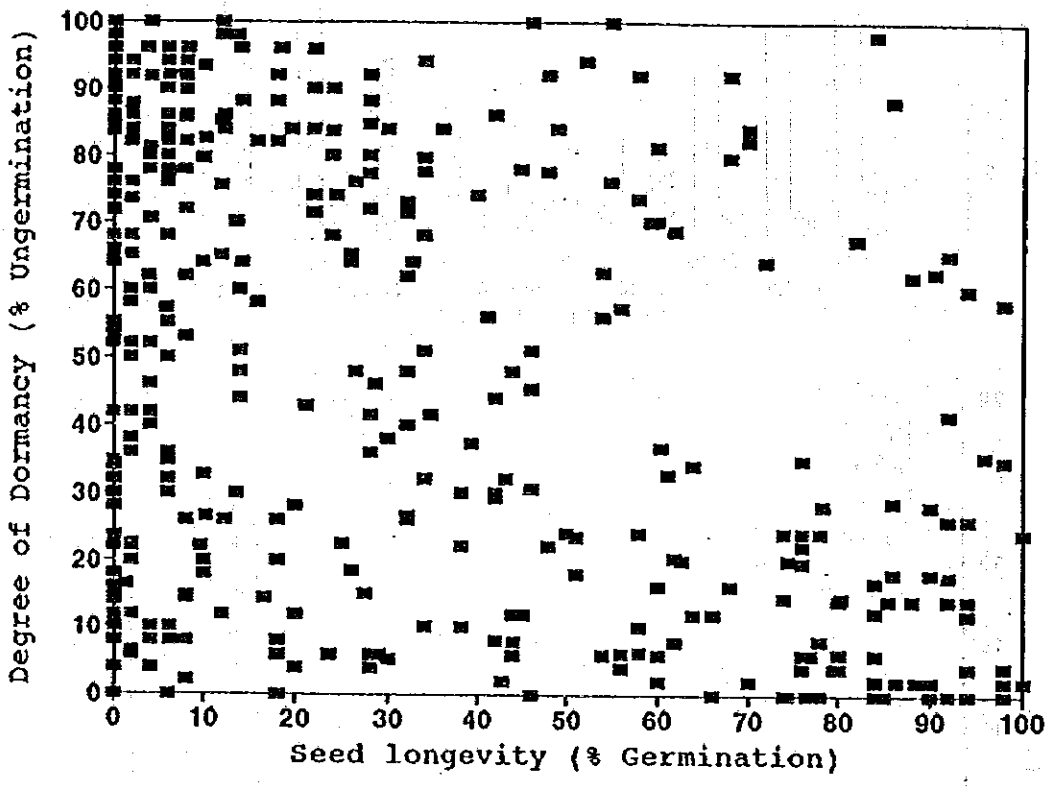

Fig. 6. Relationship between seed longevity and degree of dormancy (% ungermination seeds at 10 days after sowing in control plots) in all rice varieties.

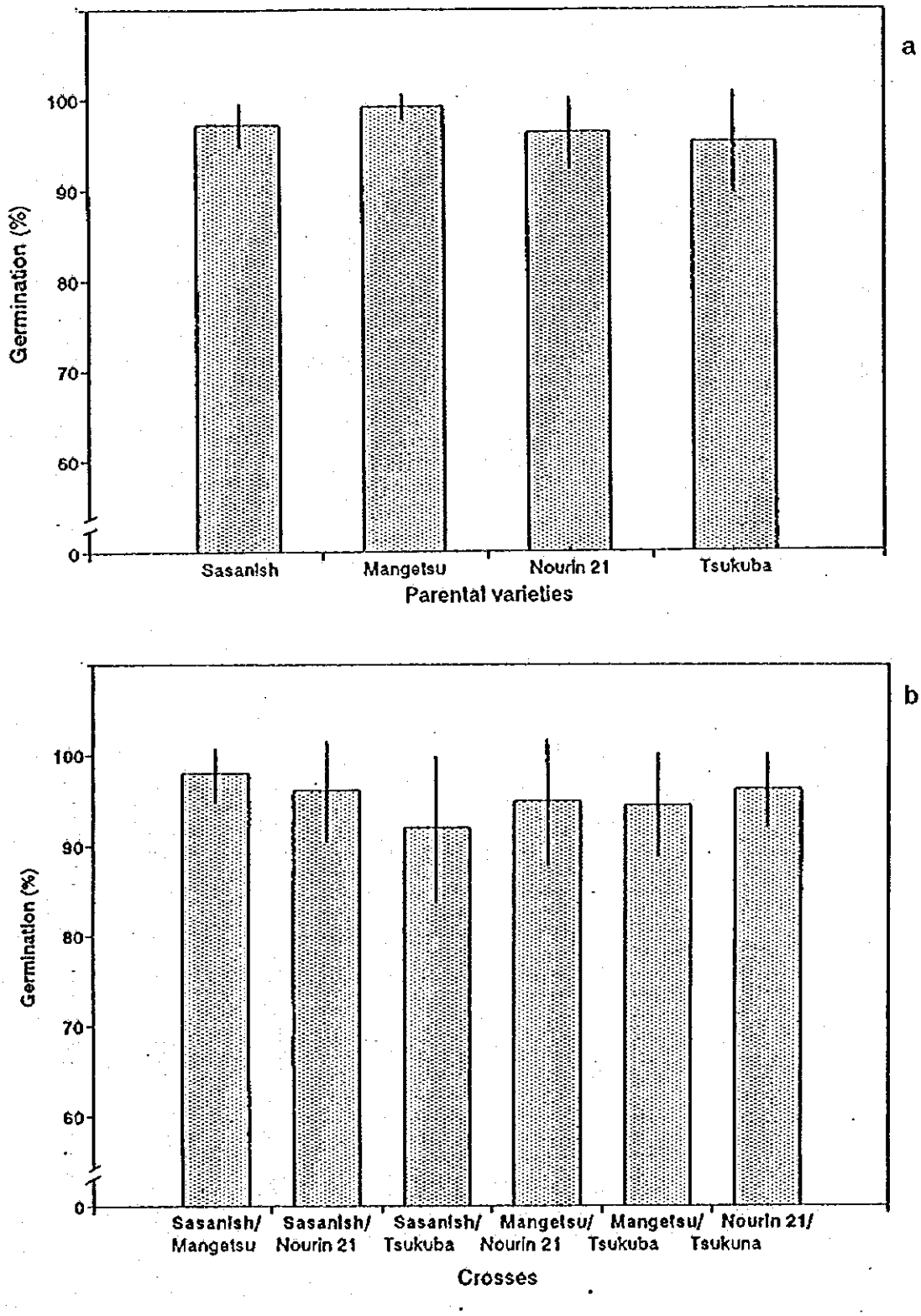

Fig. 7. Germination percentage of parental varieties (a) and crosses (b) between upland and lowland rice varieties. Average ± SD.