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KARYOTYPIC STUDIES ON SOME GENOTYPES OF HULL-LESS BARLEY (*HORDEUM VULGARE* L.)

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ABSTRACT

Karyotypic studies were carried out on 16 hull-less barley (*Hordeum vulgare* L.) genotypes, using squash technique and Aceto-orcein staining method. Chromosomal parameters examined were as follows: long arm (L), short arm (S), total chromosome length (TL), arm ratio (AR), r-value (S/L), form percentage of chromosome (%F), Chromosome volume, relative length of chromosome (%RL) and the number of satellites. ANOVA[1] indicated high significant differences for all karyotypic parameters. Genotypes tested were diploid (2n=2x=14). Satellite numbers were differed, ranging from 1 to 2 pairs and were differed in satellite length. The most chromatin length were detected in G9 (73.37 µm) while G15 demonstrated the least (30.85 µm). The types of chromosomes were determined as m in all genotypes, using Levan's chromosome nomenclature. Karyotypes were classified in 1A of Stebbin's classification. In addition to this, to test the karyotypic symmetry in more detail, other parameters, *e.g.* Romero-Zarco, total form percentage of karyotype (%TF), symmetry index (%S), coefficient of variation (%CV), dispersion index (DI) were also considered. For instance, in Romero-Zarco method, the A1 and A2 coefficients were 0.37 (G2) and 0.46 (G9), respectively. The first 3 principal component analysis PCA justified %94 of the total variations correlation determined for cytogenetical parameters. Cluster analysis was carried out for either chromosomal parameters classifying in 3 classes.

Keywords: Cytogenetic variation, Karyotype, Chromosome, Hull-less Barley, Zabol. Principal Component, Classify, Chromayin, Romero-Zarco method.

INTRODUCTION

Barley is a cereal belonging to the family Poaceae, the tribe Triticeae, and the genus Hordeum (Kremer and Ben-hammouda, 2009). Barley is the basic grain used in alcoholic beverage production and livestock feed (Szczodrak et al., 1992). The first report about hull-less barley was in Canada that Scout variety was introduced (Liu et al., 2001). Canada is leading ethanol producer and the major source of published information on hull-less barley. Several two and six-rowed cultivars of hull-less barley have been registered that Two-rowed has a lower protein content than six-rowed. Hull-less barley include the waxy and zero amylose types (Bhatty, 1997; Bhatty and Rossnagel, 1997). Hull-less is an excellent source of complex carbohydrates for human foods as a source of dietary fibre, for the preparation of food malt and production of ethanol (Bhatty, 1997; Bhatty, 1999).

Cytogenetic studies of hull-less barley are in general limited. Study of genetic and cytogenetic variation between parents to create unique gene combinations with similar chromosome morphology is important for hybridization between genotypes.

The first study of karyotype description and number of chromosome were reported by Kihara (1992) and numerical of seven chromosomes on barley offered by Lewitsky (1931). Another research showed significant difference between length and volume parameters of chromosomes and the chromatin length were detected in 46.02 to 67.23 µm (Ramesh et al., 1998). The karyotype formula of diploid, tetraploid and hexaploid of Elytrigia were determined in another research (Pei-sheng et al., 2010). Intermedia was 2n = 2x = 14 =6 m + 6sm + 2st, 2n = 4x = 28 = 2M + 10m + 16sm and 2n = 6x = 42 = 4M + 18m + 20sm respectively. Chromosomal parameters examined were as follow: long arm (L), short arm (S), total chromosome length (TL), arm ratio (AR), r-value (S/L), form percentage of chromosome (F %), chromosome volume and the number of satellites. these studies reported by previous researchers (Verma, 1980; Gennur et al., 1988 ; Verna et al.,1991; Sheidai, 2000; Mirzaie-Nedoushan et al., 2000; Bakhshi Khaniki and Ebrahim, 2000).

Therefore, studies of chromosome diversity would be helpful to understanding the details of their cytogenetic variation and promoting the utilization in the crop breeding.

MATERIALS AND METHODS

Sixteen hull-less barley populations were sampled or this study (Table 1).

Table 1. Hull-less barley taxa.

Genotype code	Identification Codes of International Collection
G1	ALISO/C13909.2//FALCON-BAR/3/HIGO…
G2	CERRANJA/3/ATACOL/ACHIRA//HIGO
G3	BF891M-611
G4	CERRANJA
G5	ZABOL LOCAL VARIETY
G6	ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI
G7	BF891M-616
G8	HINIA/HB602/3MOLA/SHYRI//ARUPO*
G9	CERRANJA
G10	SACUE/GRANDO//LINO
G11	BF891M-609(Sel;1AO)
G12	ICNB93-369
G13	FLORIPONDIO/ALDE/4/CEDRO//MATNAN/EH165/…
G14	CM67-B/CENTENO//CAM-B/3/ROW906.73/4/…
G15	PETUNIA 2
G16	ALISO/C139092/FALCON-BAR/3/HIGO

The seeds were stored at 4°C until used for study. Seeds from each population were used for the genome size, base composition and cytological analysis. For germination, seeds were put on wet Whatman paper and then placed at room temperature for several hours. Emerging root tips from 30 to 40 hours old germinated seeds were used for cytogenetic investigations. To observe metaphase plates, root-tip meristems were immersed in 0.002 M 8- Hydroxyquinoline at room temperature for 3 hours and then fixed in ethanol: acetic acid (3:1, v/v) at 60°C for 15 min, and finally stored in 70% ethanol at 4°C. To observe metaphase chromosomal, meristematic tissue was stained with 0.02% (w/v) Aceto carmine. For staining, root-tip was put on Acetocarmine at room temperature for 3 to 4 days. Roots were then gently squashed in a drop of 45% acetic acid. For the cytological investigation, Images were captured with a BX50 Olympus camera. For numerical karyotype analysis, chromosomes from five metaphase were measured for hull-less barley were identified and ordered according to their long length, short length, total length (TL), Formed percentage (F% = short arm length of desired chromosome / total length of all chromosomes × 100), the ratio (r) between the long and short arms, the ratio (R) between the longest and the shortest chromosome pair and chromosome volume. Ideograms were drawn from mean values, and chromosome types were determined according to Levan et al. (1964) and for study symmetric according to Stebbins (ST) and Romero-Zarco method Karyological features were evaluated as number of satellite pair, length chromatin of karyotype, total form percentage where %TF=(total of short arm length of all chromosomes in one karyotype / total length of all chromosomes in same karyotype × 100), the relative length (RL) of each chromosome (total length of each chromosome/ total length of all chromosomes), the global asymmetric index (ASI) where SI%=(long arms/total length of all chromosomes×100), Difference of range of relative length (DRL; =$\%RL_{Max}$- $\%RL_{Min}$) and Dispersion Index.

Grouping chromosomal parameters were performed by using by average linkage group (UPGMA) methods. Also ordination performed on the first two principal components axes (PCA). Multivariate statistical analysis was performed on standardized data (mean=0, variance=1) using SAS, Minitab and SPSS. In order to determine the most variable on the genotype studied, principal components analysis (PCA) was performed. Also correlation determined between genotypes cytogenetical parameters. Correlation analysis was performed on normal data.

RESULTS

The aim of Cytogenetic investigation is to study the cellular chromosome compliment representation of the nuclear genome. ANOVA indicated high significant differences for all karyotypic characteristics.

The chromosome content or "karyotype" is classified of both chromosome number and morphology. Details of karyotypes are presented in Table 2.

Table 2. ANOVA table of chromosomal parameters in hull-less barley

S.O.V	DF	Mean Squares						
		L	S	TL	AR	r-value	F%	Volume
Genotype	15	6.24^{***}	0.119^{***}	0.789^{***}	0.219^{***}	0.033^{***}	0.009^{***}	29.68^{***}
Chromosome	6	155.54^{***}	2.239^{***}	19.289^{***}	0.175^{***}	0.264^{***}	2.243^{***}	518.82^{***}
Genotype*chromosome	90	0.81^{***}	0.018^{***}	0.074^{***}	0.024^{***}	0.036^{***}	0.018^{***}	2.62^{***}
Error	448	0.15	0.002	0.013	0.002	0.002	0.001	1.001
Total	559							
CV%		10.75%	7.68%	4.6%	4.61%	7.03%	4.18%	7.61%

^{ns} no significant , * , ** , *** significant in α=0.05, α=0.01 and α=0.001 respectively

In all metaphase plates analyzed in root tips in hull-less barley, we observed a diploid chromosome number of 2n=2x=14 (X=7). Metaphase chromosomes pictures of genotypes maybe included. Chromosome counts were various in this genus and can be used in its taxonomy (Figs 1-16). As viewed previously the basic chromosome number some species of barley was diploid and tetraploid (2n=2x=14 and 2n=4x=28) (Mohanty et al., 1991; Ahsan et al., 1998). The basic chromosome number is of importance to determine the systematic position of a taxon at high taxonomic levels (Raven, 1975). Satellite numbers was differed, ranging from 1 to 2 pairs that differed in length it (Table 2). The

chromosomes of the species studied were of m (centromere at median region) (Table 2). Morphometric data concerning metaphase karyotypes are presented in Table 2. Chromosome length varied from 78.28 to 37.59 µm that the most chromatin length were detected in genotype No. 1 while genotype No. 8 demonstrated the least. Coefficient of variation was varied from % 46.05 (in genotype No. 9) to %14.46 (in genotype No. 15). This result determined that genotype No. 13 was more symmetric and wild genotypes of them and influence environment were lower and total form percentage (%TF) were high that determined high systematic position. Also symmetric position determined by difference of form percentage and experiential in genotype No. 13 it was high (6.50). Therefore symmetric karyotype was lower and difference of form percentage in genotype No. 9 it was lowest (5.36), therefore symmetric karyotype were high of them (Table 3).

Table 3. Karyotypic details of hull-less barley taxa studied.

No.	2n	L	S	TL	V	AR	r-value	RL	F	SA	ST	TF	DI	CV	A-Z		KF
															A1	A2	
1	14	3.95	2.95	6.89	2.41	1.34	0.76	14.29	6.11	1	1A	42.75	1.71	0.25	0.24	0.25	7m
2	14	3.29	2.08	5.37	3.33	1.60	0.63	14.29	5.55	1	1A	38.82	1.28	0.30	0.37	0.30	7m
3	14	3.50	2.79	6.29	6.10	1.25	0.82	14.29	6.33	1	1A	44.34	1.63	0.27	0.18	0.27	7m
4	14	4.68	2.78	8.46	6.15	1.29	0.79	14.29	6.38	1	1A	44.68	1.43	0.31	0.21	0.31	7m
5	14	3.42	2.58	6.01	7.48	1.33	0.76	14.29	6.14	1	1A	43.00	1.25	0.34	0.24	0.34	7m
6	14	4.86	2.99	7.85	8.65	1.62	0.62	14.29	5.44	1	1A	38.08	1.07	0.36	0.37	0.36	7m
7	14	3.66	2.48	6.14	4.47	1.48	0.68	14.29	5.78	1	1A	40.43	1.26	0.32	0.32	0.32	7m
8	14	4.34	3.62	7.96	6.72	1.21	0.83	14.29	6.50	1	1A	45.48	2.16	0.21	0.17	0.21	7m
9	14	4.52	3.64	8.15	2.84	1.24	0.81	12.02	5.36	1	1A	44.60	0.97	0.46	0.19	0.46	7m
10	14	4.57	3.68	8.25	11.51	1.26	0.80	14.29	6.37	1	1A	44.57	1.20	0.37	0.20	0.37	7m
11	14	3.36	2.78	5.14	8.89	1.33	0.76	14.29	6.14	1	1A	42.97	2.02	0.20	0.28	0.20	7M
12	14	4.77	3.64	8.23	4.29	1.37	0.73	14.29	6.01	1	1A	42.06	2.30	0.18	0.27	0.18	7m
13	14	3.00	2.47	5.47	2.36	1.21	0.83	14.29	6.45	1	1A	45.16	2.30	0.18	0.27	0.18	7m
14	14	3.26	2.57	5.83	5.56	1.27	0.80	14.29	6.30	1	1A	44.10	2.41	0.19	0.17	0.19	7m
15	14	2.53	1.87	4.41	2.73	1.33	0.77	14.29	6.07	1	1A	42.50	3.02	0.15	0.20	0.15	7m
16	14	3.44	2.46	5.90	4.30	1.41	0.72	14.29	5.59	1	1A	41.67	1.59	0.26	0.28	0.20	7m

L=longest chromosome; S=shortest chromosome; TL=total chromatin length; V=mean chromosome volume; L/S= mean long/short arms; r-value = mean longest/shortest chromosome; RL= mean relative length; F= mean form percentage (%); SA=No. satellite-chromosomes; ST=Stebbins' class; TF=mean total form percentage; DI= Dispersion Index; CV=Coefficient of variation; R-Z= Romero-Zarco; KF=karyotype formula.

Correlation coefficients are indicating relationship between characters. The correlations among various traits of cytogenetic are shown in Table 3. In chromosomal parameters, total chromosome length higher significant correlated positively with short arm. Long arm high significant correlated with total chromosome length, arm ratio high significant correlated positively with chromosome volume, r-value high significant correlated positively with form percentage, arm ratio correlated positively with long arm and form percentage correlated positively with relative length. When cytogenetic correlation between numbers of traits is significant, showing that do not high variation of genotyps from these characters. When cytogenetic correlation are not significant, showing high variation of genotypes from this characters and those population can be used in next studies for example behaviors chromosomes during mitosis.

Principal components analysis of karyological data showed that the first three components and in PCIII correlation possessed %0.94 from variation (Table 4). This was also important in variation that observed.

Table 4. Simple correlation coefficients of chromosomal parameters in hull-less barley

Parameters	L	S	Volume	TL	AR	r-value	RL	F%
L	1							
S	0.90***	1						
Volume	0.51*	0.48 ns	1					
TL	0.90***	0.99***	0.49 ns	1				
AR	0.50*	0.46 ns	0.97***	0.48 ns	1			
r-value	-0.13 ns	0.30 ns	-0.03 ns	0.30 ns	-0.07 ns	1		
RL	0.01 ns	-0.11 ns	0.33 ns	-0.05 ns	0.41 ns	-0.26 ns	1	
F%	-0.03 ns	0.25 ns	0.28 ns	0.30 ns	0.32 ns	0.63**	0.57*	1

Cluster analysis of karyological data and ordination of taxa on the first two PCA axes are presented in Figures 3 and 4. The cluster analyses make up the third major cluster, indicating third genotypes distinctness. Grouping obtained from ordination of taxa based on the first two PCA axes supports the clustering results (Figure 2,3).

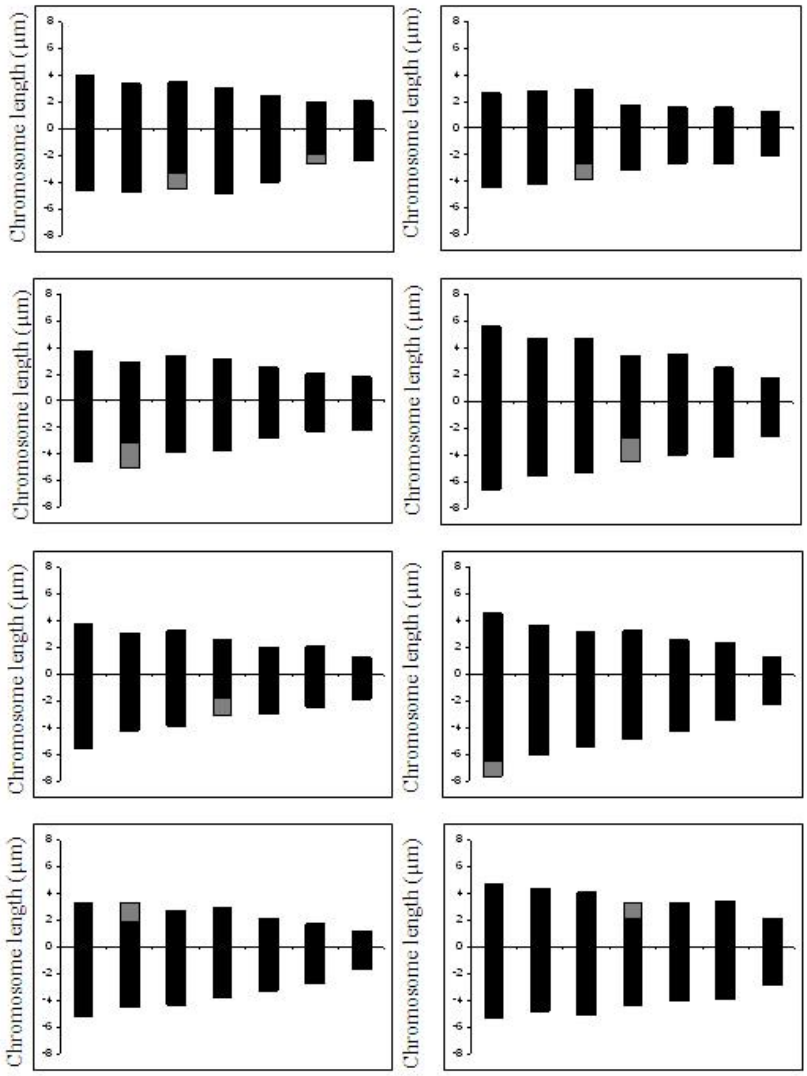

Figure 1. Ideograms of 1-8 hull-less barley.

It has been suggested that asymmetrical karyotypes are more advanced than symmetrical ones (Stebbins, 1973) and that the changes in symmetry are usually associated with chromatin loss. Among the species with 2n=14, moving from class 1A (symmetrical class). Dispersion Index indicated symmetry, respectively, were as follow: G13, G12, G11, G8, G1, G14, G3, G15, G4, G2, 7, G5, G10, G6 and G9 (Table 2).

DISCUSSION

Karyotype in hull-less barley taxa studied is symmetric. The karyotype formula was 2n=2X=14=7m. Yazdanseta et al. (2004) reported that the karyotype formula for hull-less barley was 7m which the results were the same as this experiment. The number of chromosomes were same and karyotype formula of germplasms were also same therefore the chromosomes of the species studied were centromere at median region. The chromosome constitution was uniform among different genotypes of hull-less barley. Results in this study showed evolutionary and adaptively is primary in all karyotypes and was ordered as 13 < 15 < 12 < 14 < 11 < 8 < 1 < 16 < 3 < 2 < 4 < 5 < 6 < 7 < 10 < 9. In order to check association between the change in chromosome number, total and mean chromatin length, the coefficient of correlation was showed significant association. Therefore, it may be suggested that during species diversification, the change in genotypes via structural changes has occurred mainly lown. Mirzaie-Nadoushan et al. (2000) reported difference of relative length in populations of *Bromus tomentellus* were from 2.4 to 3.5. All genotypes were classified 1A of Stebbins karyotype classification, indicated the symmetric karyotypes in genotypes.

The study revealed cytogenetic differences in ANOVA for karyological date, correlation coefficient, principal

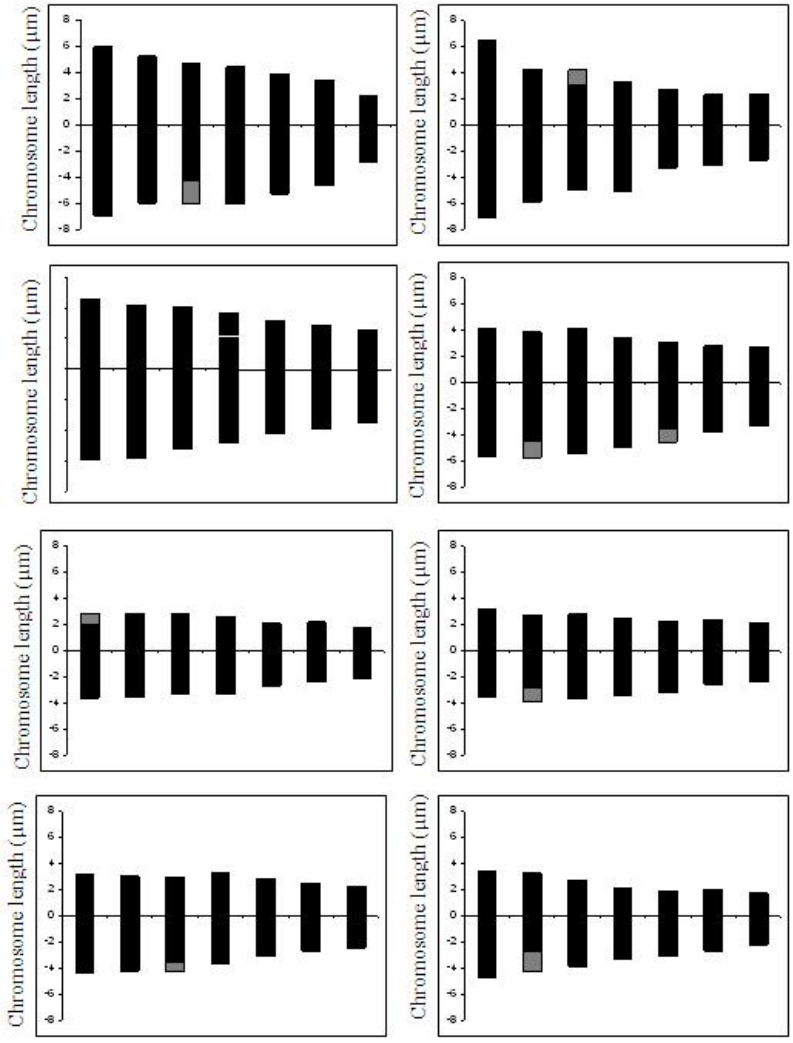

Figure 2. Ideograms of 9-16 hull-less barley.

Table 5. Correlation and coefficient determination for tree principals components of chromosome parameters in

Parameters	$\sum R^2$	r		
		PCI	PCII	PCIII
L	0.99	0.70**	0.70**	-0.04 ns
S	0.99	0.96**	0.22 ns	-0.12 ns
TL	0.99	0.86**	0.50*	-0.08 ns
F%	0.96	0.68**	-0.68**	-0.19 ns
AR	0.95	-0.68**	0.69**	0.11 ns
r-value	0.98	0.66**	-0.73**	-0.11 ns
RL%	0.97	-0.4A8**	0.30 ns	-0.80**
Volume	0.90	0.76**	0.55*	0.12 ns

component analysis and cluster analysis. Almost correlation among parameters r-value, RL and F% showed no significant, this result showing that these traits would be an effective tools to evolutionary process of genotypes as influenced that some genotypes have been away from each other. Therefore, the results of this study proposed that selective genotypes having the most homology in chromosomal variations. For this aim, crossing would permit

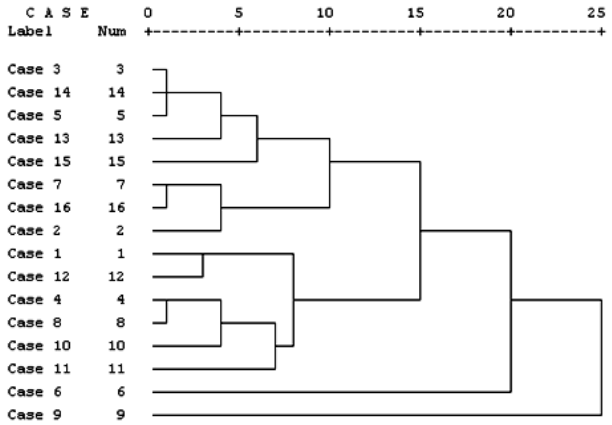

Figure 3. Phylogenic of two obtained by cluster analysis using specimen 16 No. 1 to 16 correspond to specimens in Table 1.

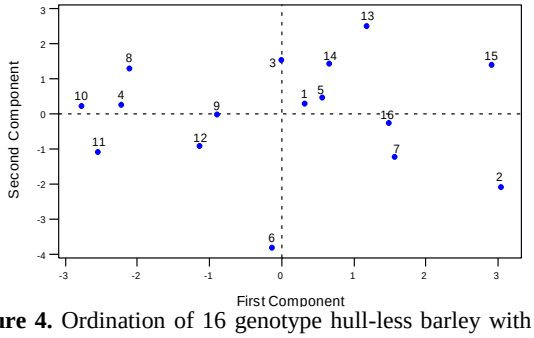

Figure 4. Ordination of 16 genotype hull-less barley with basic of chromosomal parameters karyotype.

an effective selection for crossing by genotypes 3, 14, 5, 13, 15, 7, 16 and 2 together and genotypes 1, 12, 4, 8, 10 and 11 together. Cross would not be suggestion between G6 and G9 with other genotypes because there are least homology in chromosomal variations. Recurrent selection should be more effective for improving yield might be improved by simultaneous selection the best genetic variation and the most chromosomal homology.

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