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Combining Ability Studies for Seedling Traits in Maize (*Zea mays L.*) under NaCl Stress

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Abstract. The combining ability of the major salinity tolerance traits were analysis in maize under NaCl stress. 25 hybrids were designed through incomplete diallel cross which derived from 5 salt-tolerant and 5 salt-sensitive maize inbred lines. The germination rate, height of shoot, length of root, the fresh weight and dry weight of shoot and root, the salt tolerance index (STI), the chlorophyll in seedling leaves, the proline in shoot, the K^+ and Na^+ in shoot and root to combining ability and genetic parameters were analyzed under 7‰ NaCl stress in maize. A691Ht and 21836 are screened which inbred lines' GCA are better, the A691Ht×21836, A691Ht×D49-253, 07-115×D153-70, D141-261×D80-366 and D158-20×B73 SCA are better. It was initially identified that the dry weight and fresh weight of root, the value of tolerance to salt stress, the K^+ and Na^+ in root of the genetic expression is mainly additive allelic effect.

Introduction

Maize is the main crop for both grain and forage. With the rapid development of animal husbandry, maize has become one of the main crops in saline areas in tianjin.Salt-tolerant maize varieties were planted which was one of the economic and effective methods to make full use of saline-alkali land. Although corn has salt sensitive, corn with salt tolerance can grow in severe saline and alkaline land and obtain high yield. Therefore, selecting salt-tolerant maize varieties and the in-depth study of the genetic mechanism of maize salt-tolerant traits can provide basic materials and theoretical basis for the breeding of maize salt-tolerant inbred lines and the combination of salt-tolerant maize hybrids.

Combining ability is not only an index to measure the mating ability of hybrid parents, but also a basis to select parents. To study the combining power of parents, it is very important to select parents according to the combining power for the selection of strong dominant combination[1]. Eight maize inbred lines were analyzed by Yu qiu hai et al. such as spike length, weight per ear, yield and drought resistance related properties such as hundred grain weight under water stress at the ground in the rain and the GCA, SCA and genetic variables, spikelets showed that ear length, and line number, number of grains per ear and single grain production is given priority to with gene additive effect, coarse, hundred grain weight is given priority to with the additive gene effect, also chose four useful parent and eight drought resistance, high yield and good comprehensive traits hybrid combinations[2].The results showed that the wheat salt tolerance cultivar chadianhong contained a main effective salt tolerance gene[3]. Salt tolerance of barley under salt stress is controlled by complex polygenes[4]. Shao guihua et al. found that the salt tolerance of soybean was controlled by a pair of genes, the salt tolerance was dominant, the salt sensitivity was recessive, and the salt tolerance gene was nuclear[5]. Shen fafu et al. showed that salt tolerance of cotton had additive and dominant effects through two-row hybridization analysis of salt tolerance, mainly additive effect, and salt tolerance was not completely dominant and controlled by a pair of main genes[6]. Sajjad found that the salt tolerance of maize also had additive and dominant effects[7].

Salt tolerance of maize is a quantitative trait controlled by multiple genes.The research on the genetic law of maize salt-tolerance mainly focuses on the selection of maize salt-tolerance varieties and different genotypes.It have been fewreported on the analysis of the genetic and general combining ability of maize agronomic traits under salt stress. According to previous studies, incomplete double-row hybridization design can be adopted to study the selection method of strong dominant combination by measuring plant growth status, physiological and biochemical indexes such as ion, chlorophyll and proline, and calculating the combining ability of these related salt-tolerant traits, so as to provide theoretical basis for the hybridization breeding of salt-tolerant maize.

Experimental Materials and Methods

Experimental Materials

Maize Varieties

5 salt-tolerant maize inbred lines A691Ht, D80-366, Longyu113(♂), 07-115, 21836 and 5 salt-sensitive maize inbred lines B73, D141-261, D158-20, D153-70, D49-253 and their incomplete diallel cross combination.

Reagent

Distilled water; 7‰ NaCl Solution; Hoagland solution; 0.1%mercuric chloride solution; 95% ethanol solution; calcium carbonate; quartz sand; acid indene ketone solution three; 3% sulfo salicylic acid solution; Toluene; acetic acid; hydrochloric acid solution of 2mol/L.

Instrument

Germination box, flask, glass rod, volumetric flask, electronic balance, oven,incubator, ultraviolet visible spectrophotometer, ion spectrophotometer.

Experimental Methods

Pretreatment of Seeds

Maize hybrid seed with the same grain plumpness, size, shape and maturity was selected from the maize varieties of experiment, 180 grain per variety. Disinfection by 0.1% $HgCl_2$ solution of 15 min then wash cleanly with rinsing, prepared for the experiment.

Seed Culture

Culture with germination box in the laboratory. The pretreated maize seeds were sowed in germination box with 600g sand, 25 grains per germination box. Experiments repeated 3 times. 2 germination box of every repetition were poured into the 120mL Hoagland solution and 120mL 7‰ NaCl Hoagland solution. Among them, the germination box that only with Hoagland solution as a control group, represented by CK, adding 7‰ NaCl are represented by 7‰.

Germination Experiment

Prepared germination box were cultured in incubator,12 hours of light, 12 hours of darkness a day, a constant temperature at 25°C, relative humidity at 75%.

Determination of the Project

Germination rate: germination rate of maize seeds were determined in the seventh day during culture, by standard of 1cm shoot. germination rate=(total number of shoots in 7days/total number of seeds)×100%.

Height of shoot, length of root:Harvesting the seedlings in the twenty-first day, 5 seedlings which the growth is uniform were measured for their seedling height and root length and record respectively.

Shoot dry weight, root dry weight: the fresh weight was measured, then the shoot and root were put into the aluminum box, 1h in an oven at 105°C, 80°C drying 24h. The dried shoots and roots were weighed to get shoot dry weight and root dry weight.

Salt tolerance index: Salt tolerance index = (7‰ seedling dry matter accumulation/CK dry matter accumulation)×100%

Chlorophyll content in leaves: use scissors clipping 0.2g fresh leaves of maize seedlings, into the mortar, adding 10mL 95% ethanol solution, a little quartz sand and calcium carbonate powder, ground for 3-5 minutes. The research used filter paper to 25mL Brown volumetric flask, constant volume, shake. By using UV visible spectrophotometer, using 95% ethanol solution as a blank at the wavelength of 665nm, 649nm, 470nm, determination of absorbance.

Proline content: use scissors clipping 0.5g fresh leaves of maize seedlings, in a test tube, add 5mL 3% sulfo salicylic acid, extraction of 10min (in a boiling water bath extraction process always shake), after cooling to 3000r/min centrifugal 10min, 2mL with the supernatant from test tube is to be measured. With 2 acetic acid and three 2mL acid indene ketone solution in a test tube, the tube is placed in a boiling water bath for 30min after cooling to room temperature, adding 4mL toluene, shake after holding 0.5min, stay after stratification by micropipette upper proline red toluene solution in the cuvette, using toluene as the blank control, determination the absorbance at a wavelength of 520nm, proline concentration values found in the standard curve, converted to proline content in the samples.

The aboveground and underground parts of K^+, Na^+ content: stem, root samples fixed and dried separately grinding into powder, called stem sample powder 0.50g, 0.20g root sample powder, respectively, into the crucible, carbonation in EAF in muffle furnace 550°C ashing 8h. Using HCl ash dissolved in 2mol/L, set the volume to a 25L volumetric flask, measure K+, Na+ ion content by flame spectrophotometer.

Results and Analysis

Variance Analysis of Combining Ability

Table 1. Analysis of variance of trait (F value).

Sources of variation	Area group	Combination	Among female parents	Among male parents	The parents of interaction
degree of freedom	2	24	4	4	16
Germination percentage	1.09	10.11**	1.15	1.14	9.64**
Height of seedling	1.06	6.59**	1.78	2.36	4.85**
Root length	0.56	7.89**	3.02*	1.54	5.53**
Seedling fresh weight	0.83	20.65**	0.9	2.14	17.59**
Root fresh weight	0.7	5.53**	4.52*	0.71	3.59**
Seedling dry weight	1.98	1272.11**	1.09	0.77	1304.15**
Root dry weight	1.05	16.67**	3.59*	1.01	11.63**
Salt tolerance index	0.12	15.55**	6.20**	1.75	7.81**
Chlorophyll	2.94	1091.22**	0.91	1.24	1065.17**
Proline	0.19	478.73**	1.56	0.06	510.87**
Aboveground part of potassium ion content	0.66	290.70**	1.33	1.39	259.47**
Underground part of potassium ion content	1.36	557.38**	6.45**	7.13**	190.25**
Aboveground part of sodium ion content	2.57	233.47**	2.06	0.36	218.06**
Underground part of sodium ion content	0.31	580.25**	3.17*	1.58	397.89**

Table 2. The trait value of general combining ability in inbred lines.

Inbred lines	A691Ht	Longyu113 (♂)	07-115	D141-261	D158-20	D80-366	21836	B73	D153-70	D49-253
Germination percentage	6.93	-7.66	-8.76	4.01	5.47	-1.82	9.12	5.11	-10.58	-1.82
Height of seedling	-1.9	-2.22	-1.98	8.98	-2.88	0.76	8.86	-6.48	0.52	-3.66
Root length	11.71	1	-18.46	6.06	-0.31	0.29	11.46	-1.69	1.25	-11.31
Seedling fresh weight	-10.23	4.49	-0.06	4.42	1.38	3.21	13.1	-8.07	1.65	-9.89
Root fresh weight	10.78	-9.7	-24.37	10.64	12.65	-5.8	8.39	-1.78	5.19	-6
Seedling dry weight	-28.06	104.95	-26.15	-26.71	-24.03	-26.07	-11.78	-24.48	-25.12	87.46
Root dry weight	4.81	-9.01	-22.26	8.12	18.34	0.21	13.65	-8.73	-0.79	-4.35
Salt tolerance index	-4.33	-6.75	25.61	-15.97	1.45	11.05	6.07	-2.61	-5.67	-8.84
Chlorophyll	1.69	4.22	-9.69	-3.79	7.58	7.04	-12.28	-1.67	-0.15	7.07
Proline	38.58	8.05	-40.63	-2.08	-3.91	6.53	-8.61	-1.42	0.92	2.58
Aboveground part of potassium ion content	-12.26	-0.42	2.71	16.19	-6.22	5.65	-12.31	-11.46	9.94	8.18
Underground part of potassium ion content	-28.05	-1.81	11.6	34.55	-16.29	-4.65	-29.43	-17.92	19.82	32.18
Aboveground part of sodium ion content	-25.16	7.65	4.94	2.48	10.1	-2.33	1.96	-8.05	-0.05	8.47
Underground part of sodium ion content	-17.22	-1.26	-0.9	-1.67	21.04	6.38	-2.77	-14.56	0.38	10.56

Table 3. The trait SCA effects of 25 combinations.

Combination	**Germination rate**	**Seedling height**	**Root length**	**Seedling fresh weight**	**Root fresh weight**	**Seedling dry weight**	**Root dry weight**
A691Ht×D80-366	-6.40	4.19	4.58	6.73	-12.87	31.41	-8.12
A691Ht×21836	-3.26	-8.61	5.72	-14.95	10.58	18.49	27.36
A691Ht×B73	-0.70	-2.48	-0.05	-0.21	26.67	15.22	-13.87
A691Ht×D153-70	-9.31	1.98	-12.01	1.01	-17.73	32.07	-6.34
A691Ht×D49-253	19.67	4.92	1.75	7.41	-6.64	-97.20	0.98
longyu113(♂)×D80-366	-2.36	13.31	26.69	20.87	14.52	-81.62	13.20
longyu113(♂)×21836	12.22	-10.13	-21.14	-16.26	-16.51	-112.99	-27.13
longyu113(♂)×B73	-7.88	0.51	-6.78	-13.07	-21.22	-104.15	-5.37
longyu113(♂)×D153-70	5.00	-7.10	6.50	-0.95	7.00	-102.55	1.23
longyu113(♂)×D49-253	-6.98	3.41	-5.28	9.41	16.21	401.31	18.07
07-115×D80-366	9.88	-13.11	2.30	-10.36	-10.46	17.71	-2.46
07-115×21836	-43.78	12.33	-14.59	20.12	5.53	38.81	11.29
07-115×B73	22.21	0.47	0.97	-15.20	-7.66	14.11	-0.87
07-115×D153-70	6.50	-6.12	16.66	11.56	2.59	27.11	-5.21
07-115×D49-253	5.19	6.44	-5.34	-6.12	10.00	-97.75	-2.74

D141-261×D80-366	10.65	-4.85	-21.92	-5.59	16.87	20.44	15.45
D141-261×21836	11.76	7.91	23.99	12.82	5.40	32.55	-7.21
D141-261×B73	-12.01	-0.68	0.49	7.88	-22.65	33.05	-19.69
D141-261×D153-70	-5.68	4.60	-9.96	-3.12	11.32	19.25	17.39
D141-261×D49-253	-4.72	-6.98	7.40	-12.00	-10.93	-105.29	-5.93
D158-20×D80-366	-11.76	0.47	-11.65	-11.65	-8.06	12.06	-18.06
D158-20×21836	23.05	-1.50	6.02	-1.74	-4.99	23.13	-4.31
D158-20×B73	-1.62	2.18	5.36	20.60	24.86	41.77	39.80
D158-20×D153-70	3.49	6.64	-1.19	-8.51	-3.18	24.11	-7.06
D158-20×D49-253	-13.16	-7.79	1.45	1.30	-8.63	-101.07	-10.37

Combination	**Salt tolerance Index**	**Chlorophyll**	**Proline**	**Potassium content of overground**	**Potassium content of underground parts**	**Sodium content of overground parts**	**Sodium content of underground parts**
A691Ht×D80-366	-15.69	3.89	-50.01	-31.34	-21.63	-4.39	-6.40
A691Ht×21836	17.80	3.57	-7.59	27.35	21.23	11.22	-3.26
A691Ht×B73	-8.60	34.14	-75.59	17.42	14.68	-13.22	-0.70
A691Ht×D153-70	-12.12	-17.27	55.21	-12.34	-4.70	-11.27	-9.31
A691Ht×D49-253	18.60	-24.33	77.97	-1.09	-9.58	17.67	19.67
longyu113(♂)×D80-366	1.73	14.03	-20.25	29.55	24.34	12.04	-2.36
longyu113(♂)×21836	1.20	-5.45	70.19	7.26	4.80	6.67	12.22
longyu113(♂)×B73	-0.30	-1.97	23.11	-1.17	12.15	-3.43	-7.88
longyu113(♂)×D153-70	2.08	-0.32	-30.53	-22.93	-15.78	-7.21	5.00
longyu113(♂)×D49-253	-4.71	-6.28	-42.52	-12.70	-25.52	-8.07	-6.98
07-115×D80-366	11.39	-18.00	54.31	-6.79	13.65	43.53	9.88
07-115×21836	-3.35	-9.92	-23.40	-31.04	-42.42	-47.82	-43.78
07-115×B73	-5.08	-2.00	-22.59	7.52	0.59	10.36	22.21
07-115×D153-70	9.10	11.68	4.01	24.76	7.54	-5.30	6.50
07-115×D49-253	-12.06	18.24	-12.34	5.55	20.64	-0.76	5.19
D141-261×D80-366	20.43	3.19	15.69	13.48	-9.31	-37.00	10.65
D141-261×21836	-5.00	-2.19	-19.20	-5.94	-2.31	16.23	11.76
D141-261×B73	-7.29	-14.23	-4.70	-29.42	-25.09	7.36	-12.01
D141-261×D153-70	-9.60	1.20	24.30	11.28	11.41	21.40	-5.68
D141-261×D49-253	1.46	12.03	-16.09	10.59	25.29	-7.99	-4.72
D158-20×D80-366	-17.86	-3.11	0.25	-4.90	-7.05	-14.17	-11.76
D158-20×21836	-10.65	14.00	-20.00	2.37	18.70	13.69	23.05
D158-20×B73	21.27	-15.94	79.77	5.65	-2.33	-1.06	-1.62
D158-20×D153-70	10.54	4.71	-53.00	-0.77	1.52	2.38	3.49
D158-20×D49-253	-3.30	0.34	-7.02	-2.36	-10.84	-0.84	-13.16

Variance analysis of randomized trials (Table 1) showed that: the characters,the interaction between the parents were extremely significant; general combining ability effects between parents significantly on root length, root fresh weight, root dry weight and the underground part of the content of sodium ion, the index of salt tolerance, the influence of content of underground parts of potassium ion highly significant, effect on other traits were not significant; the influence of content of general combining ability effect to the underground part of potassium ion between male very significant, effect on other traits were not significant. Therefore,the general combining ability (GCA) of parents and specific combining ability (SCA) of parents can be estimated further more .

The Traits of Inbred Lines of GCA Effects and Analysis

GCA refers to a parent (inbred lines) the average performance for a series of crosses and the other parent group with the yield and other traits. The GCA is determined by gene additive effect, can be genetic, so, Inbred line with high GCA, can produce strong heterosis, have a great effect on hybrid offspring [8].

As can be seen from table 2 the GCA effect value, 14 characters measured effect values of GCA showed positive or negative effect, the same inbred have larger differences in GCA among different characters also, the inbred lines in different characters on gene control methods are different, the genetic effects were also different [9]. The germination rate of GCA effect value is positive relative with A691Ht, D141-261, D158-20, 21836, B73, GCA effects of seedling height value were high with D141-261, 21836, GCA effects of root length value were high with A691Ht, D141-261, 21836. 21836 of them in the germination rate and root length, height of seedling performance are very good, D141-261 in germination rate,height of seedling and root length of the performance are very good, D141-261 in germination rate and root length, height of seedling performance is good.

A comprehensive analysis of the GCA effect value can be seen, in the seedling, root dry and fresh weight, inbred lines Longyu 113 (♂), D141-261, D158-20, 21836 had good performance. Among them, 21836 in the seedling fresh weight had an outstanding performance, Longyu 113 (male) and D49-253 in seedling dry weight had an outstanding performance, D158-20 and 21836 in root dry weight had an outstanding performance.

GCA of salt tolerance index relative effect value is positive with 07-115, D158-20, D80-366, 21836, of which 07-115 is very high. GCA effect value of chlorophyll is positive with A691Ht, Longyu113 (♂), D158-20, D80-366, D49-253. GCA effect value of proline is positive with A691Ht, Longyu 113 (♂), D80-366, D153-70, D49-253, Among them, A691Ht is very high.

Aboveground and underground parts of the GCA effect value of potassium ions were positive with 07-115, D141-261, D153-70,D49-253. In addition to D80-366 aboveground parts of the GCA effect value of potassium ions were positive , the underground part of GCA effect value is negative, others were negative. A691Ht aboveground and underground parts of sodium ions are higher negative, D158-20 are higher positive. The others has no significant features.

Analysis of SCA Effects of Hybrids Combinations

Getting high SCA refers to the special effects level of hybrids that is combined with two specific parental inbred lines, which depends on the non-additive effects of genes.it is a genetic non-fixed part and greatly influenced by the environment, it can be used for guiding the utilization of heterosis and cross-breeding [10].

Each trait SCA effects of 25 combinations in this study is in Table 3. It can be seen clearly that the variation ranges of SCA relative effects.As follows, the germination rate: -43.78~23.05; seedling height: -13.11~13.31; root length: -21.92~26.69; seedling fresh weight: -16.26~20.87; root fresh weight: -22.65~26.67; seedling dry weight:-112.99~401.31 ; root dry weight: -27.31~39.8; salt tolerance index: -17.86~21.27; chlorophyll: -24.33~34.14; proline: -75.99~79.77; potassium ion content of overground parts: -31.34~29.55; potassium ion content of underground parts: -42.42~25.29; sodium ion content of overground parts: -47.82~43.53; sodium ion content of underground parts: -43.78~23.05. Salt tolerance index is an essential factor to determine the strength of the salt-tolerant maize.There are some combinations of the high SCA effects of salt tolerance index in the 25 tested varieties, which are D158-20 × B73, D141-261 × D80-366, A691Ht × D49-253, A691Ht× 21836. With comprehensive consideration of 14 Salt-related indicators, the better combinations of SCA are A691Ht × 21836, A691Ht × D49-253, 07-115 × D153-70, D141-261 × D80-366, D158-20 × B73.

Estimates of Population Genetic Parameters of Each Trait

As shown in the Table 4, SCA genotypic variances were greater than GCA genotypic variances in all traits but potassium ion content of underground parts,it indicated that these traits mainly controlled by non-additive genes, whereas, additive genes had minor effect.General combining ability (GCA) variances in sequence from high to low were: potassium ion content of underground parts, salt tolerance index, root fresh weight, root length, root dry weight,sodium ion content of underground parts, seedling height, seedling fresh weight, sodium ion content of overground parts, potassium ion content of overground parts, proline, germination rate, chlorophyll, seedling dry weight.Specific combining ability (SCA) variance decreased in seedling dry weight, chlorophyll, germination rate, proline,potassium ion content of overground parts, sodium ion content of overground parts, seedling fresh weight, seedling height,sodium ion content of underground parts, root dry weight, root length, root fresh weight, salt tolerance index ,potassium ion content of underground parts and it is just opposite with the order of GCA variances.As can be seen from Table 4, the broad-sense heritability of traits from better to less were: seedling dry weight> chlorophyll> potassium ion content of underground parts> sodium ion content of underground parts> proline> potassium ion content of underground parts> sodium ions content of overground parts> seedling fresh weight> root dry weight> salt tolerance Index> germination rate> root length> seedling height> root fresh weight.Meanwhile, in addition to potassium ion content of the underground parts, narrow-sense heritabilities of other traits are less than 55%, which indicated that these traits could be easily affected by the environmental conditions.The broad-sense heritabilities of Seedling dry weight, chlorophyll, proline,potassium and sodium contents of overground and underground parts were more than 98%, which indicated that these traits were less affected by non-genetic factors .

Discussion

Analysis of Maize Inbred Seedlings Salt Tolerant Trait GCA Effect under Salt Stress

The genetic analysis of 14 salt tolerance related traits including the germination rate, seedling height, root length, seedling fresh weight, root fresh weight, seedling dry weight, root dry weight, salt tolerance index, chlorophyll content in leaves, proline in seedlings, potassium content of overground parts, potassium content of underground parts ,sodium ion content of overground parts, sodium ion content of underground parts showed that: the interactions of the parents existed significant differences among the traits combinations; the potassium ion content of underground parts in the female parent and the male parent exist highly significant differences ; root length, root fresh weight, root dry weight, sodium ion content of the underground parts etc in the female parent exists significant difference; salt tolerance index exists highly significant differences in the female parent. After Comprehensive analysis of GCA and SCA, the initial results of the high GCA inbred under salt stress are A691Ht and 21836.

Analysis of Seedlings Salt Tolerant Trait SCA Effect in Each Maize Combination under Salt Stress

Salt-tolerant index is an essential factor of maize salt-tolerant strength in the 25 tested varieties, the combinations with high SCA effect of salt-tolerant trait are D158-20 × B73, D141-261 × D80-366, A691Ht × D49-253, A691Ht × 21836. Comprehensively considering 14 salt-related indicators, the combinations with better SCA are A691Ht × 21836, A691Ht × D49-253, 07-115 × D153-70, D141-261 × D80-366, D158-20 × B73.

Analysis of Maize Salt-tolerant Genetic Parameters under Salt Stress

The broad-sense heritabilities of various traits from better to less were: seedling dry weight>chlorophyll>potassium content of underground parts>sodium content of underground parts > Proline> potassium content of underground parts >sodium content of aboveground parts> seedling fresh weight> root dry weight> salt-tolerant index> germination rate> root length>seedling height> root fresh weight, the descending order of narrow-sense heritability were: potassium content of underground parts> salt-tolerant index> sodium content of underground parts> root fresh weight> root dry weight > root length>seedling height> sodium content of aboveground parts> seedling fresh weight> potassium content of aboveground parts> Proline> germination rate> chlorophyll> seedling dry weight.The broad-sense heritability of root dry weight, fresh weight, salt-tolerant index,potassium ion and sodium ion contents of the underground parts were more than 50%,their narrow genetic rate were over 30% ,so the preliminary judgment[21] is that main genetic expressions of root dry weight, root fresh weight, resistance index additive gene effects, with the supplement of non-additive gene effects.

Summary

A691Ht, 21836 with high GCA and ordinary salt tolerance and A691Ht × 21836, A691Ht × D49-253, 07-115 × D153-70, D141- 261 × D80-366, D158-20 × B73with high SCA have been selected in seedling stage under salt stress. It can be preliminary concluded that genetic expressions of root dry weight, root fresh weight, salt-tolerant index, potassium ions sodium ions contents of underground parts are mainly affected by additive gene and supplemented by non-additive gene ;the rest traits are determined by the non-additive genes and additive genes together, which are susceptible to environmental conditions.

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