

Combining ability and gene action studies for drought tolerance in tomato

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Abstract

Physiological traits of tomato including its resistance to stresses are a main breeding goal in producing new cultivars. This study reports on a combining ability analysis investigating the variance of general and specified combing abilities for some important physiological characteristics as a whole as well as their effects for individual parents and hybrids of 19 tomato genotypes of tomato under drought stress. Three commercial innate lines and four analyzers were used in a line-to-tester crossing plan at Ilam University, Iran. There was a significant difference between genotypes (parents and crosses) in all characteristics at three levels of stress. Evaluating the impacts of common combining capacity analyzers and lines showed that neither a single line nor an analyzer was a commendable common combiner for all of the characteristics examined at all three push levels. Estimation of the effects of specific combining ability indicated that for each specific physiological trait, a specific hybrid showed the highest effect at all three stress levels. In all of the traits under study, specific combining ability variance had a higher estimation than general combining ability variance, and the genetic variance ratio of additive variance to non-additive variance was smaller than one, indicating that nonadditive gene action predominated in the inheritance of all of the characteristics in the three levels of stress. The degree of dominance under three levels of stress was higher than one for all attributes except total soluble solids, and it seems that dominance in the genetic locations controlling these traits is superseded.

Keywords: combing ability, drought stress, gen action, line × tester, peroxidase

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Introduction

Tomato (*Solanum lycopersicum L.*) is one of the most important vegetables in Iran and is widely

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cultivated owing to its fleshy fruits. It is a warmseason vegetable all over the world with multipurpose utilization, adaptability, and high yield. Tomato fruit is considerable in terms of carotenoids, ascorbic acid, and lycopene and is highly regarded for its color, taste, and antioxidant

activity (Tilahun et al., 2021). Lycopene is said to have anti-cancer properties and is considered as a powerful common antioxidant (the most noteworthy successful singlet oxygen quencher) utilized inside pharma.

Genotypes' combining abilities indicate how well they combine with a given genotype to produce populations with potential and productivity. The idea of general (GCA) and specific combining ability (SCA) aids the breeder in selecting the parents for hybridization, isolating promising genotypes from the segregating population, and gaining knowledge of gene action, which helps to understand the principles of plant trait inheritance (Sprague and Tatum, 1942). Kempthorne's (1957) Line \times Tester mating design aids breeders by providing details on the combining ability status of the genotypes (parents) used and the kind of gene action involved. The estimation of GCA and SCA variations as well as their impacts have been considerably used in this design. Moreover, it is employed to comprehend the type of gene action responsible for the expression of economically significant quantitative characters.

Owing to various benefits of hybrids over pure line varieties in response to commercial yields of fruits and their different characteristics, the commercial exploitation of hybrid vigor in tomatoes has gained more importance. For the exploitation of heterosis, the choice of parents is of paramount importance (Mukri et al., 2020). Kumar et al. (2013) investigated a line \times tester analysis in tomato using 10 lines and three testers. They discovered that all fruit quality traits, for example ascorbic acid, lycopene (LYC), titrable acidity (TA), and total soluble solids (TSS) were controlled by non-additive gene action. Over-dominance was prevalent in the majority of the traits. Arora et al. (2022) reported the importance of additive and non-additive gene action in a line $(8) \times$ tester (32) analysis, with the non-additive gene action predominating for fruit weight and ascorbic acid content. Reddy et al. (2020) found that titrable acidity and ascorbic acid had predominant nonadditive genetic variance. According to Mondal et al. (2009), non-additive gene action governs features of fruit quality like total soluble solids and lycopene content. Katkaret al. (2012) investigated 57 F1 hybrids and discovered that the estimated

variance of GCA and SCA, as well as their ratio, indicated a predominance of non-additive gene action for total soluble solids and ascorbic acid. Mondal et al. (2009) discovered that both the additive and dominance components were highly significant, implying that both additive and dominance gene action are important for the conditioning of lycopene, total soluble solids, and *β*-*carotene* in tomato.

The current study attempted to obtain information on the amount of GCA and SCA variance for some important physiological characteristics as a whole, as well as GCA and SCA effects for individual parents and hybrids, by combining ability analysis.

Material and Method

The research was carried out at Illam University's Experimental Research Farm between 2017 and 2018. A total of 19 tomato genotypes, including three inbred lines (Bitstoik, Kingston, and Peto early), given by the Seed and four testers [LA2080 (S. lycopersicum var. cerasiforme), LA2656 (S. pimpinellifolium), LA1607 (S. pimpinellifolium), and LA1579 (S. pimpinellifolium)] were used. Initially, parental seeds were planted in the chassis. Then, the seedlings with 2-4 leaves were transferred to pots (23 x 20 cm) containing a mixture of 1:1:1 garden topsoil, leaf mold, and fine sand at the stage of 2-4 leaves. At the beginning of the flowering period, the cross between the lines and desired testers was carried out, and the seeds of the F1 generation (hybrid) were obtained. In the second year, obtained F1 and parents were evaluated at three levels of drought stress. The three drought stress levels were 100 percent field capacity (FC) (S1: control), 40% FC (S2: mild stress), and 60% FC (S3: severe stress). At each stress level, a randomized complete block design with 3 replications was used. Seeds from parents and hybrids were grown in plastic pots for two months before being transplanted to the field plot at 75 × 30 cm spacing. Water stress treatments were applied after the entire plant had been deployed in the field. Irrigation treatments are carried out in accordance with field capacity. Drought relief was maintained until the harvest. The crop was grown in accordance with the area's standard cultural recommendations. Water stress treatments were applied after the complete plant deployment in the field. Irrigation treatments were carried out according to the capacity of the farm. Drought stress treatment was carried out until harvest.

Proline measurement

Proline was determined using the reaction of proline with ninhydrin, as described by Bates et al. (1973). One gram of fresh leaves was powdered in three ml of sulfosalicylic acid 3%. The homogenized components were centrifuged and the supernatant was transferred to the tube, and 2 ml of ninhydrin and 2 ml of glacial acetic acid were added to it. The supernatant was moved to a tube, and glacial acetic acid and ninhydrin were added to it. Then, the tubes were closed before being placed in a 100 ℃ hot water bath. After the contents of the tubes were cooled, 4 ml of toluene were added to each tube which were shaken for 20 seconds. Finally, the red supernatant phase containing proline in toluene was separated and measured at 520 nm in a spectrophotometer alongside standard specimens. A standard curve was used to calculate the concentration of proline in milligrams per gram fresh leaf tissue.

PH measurement

The PH of extracts was determined with a pH meter after the extraction and filtration of extracts (a mix of 3 fruit extracts for each experimental unit).

Relative water content

These measurements were taken using a method previously described by Korkmaz et al. (2010).

Measurements of Chlorophylls

The chlorophyll (Chl) content of young and fully developed leaves was determined using fresh samples (0.1 g). The samples were centrifuged with 5 milliliters of acetone (80% v/v) after being homogenized with 5 milliliters of the solvent. Absorbance was measured at wavelengths of 663 and 645 nm, and chlorophyll content was measured using the Strain and Svec (1966) equations.

Measurements of malondialdehyde (MDA)

The TBA reaction, described by Heath and Packer (1968) was used with a few modifications to determine the lipid peroxidation index as well as the content of malondialdehyde. In this approach, 0.25 g of fresh leaf was rinsed in 5 ml of trichloroacetic acid (0.1) before being centrifuged at 10,000 rpm for 5 minutes. One ml of the upper solution was combined with 4 ml of 20% trichloroacetic acid containing 0.5 g of thiobarbituric acid, then kept on ice for 30 minutes in a 95 ℃ water bath. The supernatant was then utilized to determine MDA content spectrophotometrically. The absorbance at 532 nm was calculated by subtracting it from the absorbance at 600 nm. Lastly, the quantity of malondialdehyde was estimated using the equation below.

malondialdehyde (nmol/g Fresh Weight) = [(532 nm-600 nm) / (cuvette path length \times quenching factor)] × (Dilution Factor).

Measurement of catalase

To calculate catalase enzyme activity, leaf protein was first extracted. After protein extraction, 4.51 µl of 30% hydrogen peroxide and 3 mL of 50 µl enzyme crude extract buffer (pH = 7) were mixed in an ice bath. The curve absorbance at 240 nm wavelength was obtained after two minutes, and the results were displayed based on the μmol (H2O2)/mg protein (Aebi, 1984).

Peroxidase measurement

The method of measuring this enzyme is similar to that of catalase, but with the following differences. The extraction buffer for this enzyme contained 35.3 µl of guaiacol in a 50 mM phosphate buffer. Also, the spectrophotometer absorption wavelength was set to 470 nm. 3), and the time required to stabilize the enzyme reactions was 2 minutes.

Ascorbate peroxidase activity measurement

Ascorbate peroxidase activity was measured using Chance and Maehly (1995). The reaction composition included 4.51 μ l H2O2 (30%), 100 μ l sodium ascorbate, 50 µl crude enzyme extract,

and 1050 mM phosphate buffer (pH = 7). After the addition of hydrogen peroxide, 290 nm was used as the wavelength for measuring absorbance in 1 minute, and the data was reported as μmol H2O2/mg protein.

Soluble solid concentration measurement

Soluble solids concentration (TSS) was measured using an ATC-1E refractometer at 20±1 ℃. Initially, the refractometer was calibrated using double distilled-water, and the findings were stated as %TSS.

Titrable acidity measurement

Titrable acidity was measured using the titration method where 2.5 mL of tomato juice was mixed with 47.5 mL of distilled water and just a few drops of phenolphthalein before being titrated with 0.1 N NaOH to pH 8.2. Titrable acidity was determined by the amount of citric acid (the dominant acid in tomatoes) per 100 grams of fresh weight (Ayala-Zavala et al., 2004).

Flavonoid measurements

A sample of 0.1 g of freshly harvested tomato fruit tissue was extracted in a tube with 10 ml of acidified ethanol [ethanol: glacial acetic acid (99:1 v/v)]. Before being brought up to volume, the samples were gently cooked in an 80 ℃ water bath for 10 minutes. Testing for absorbance was done using a spectrophotometer at three different wavelengths: 270, 300, and 330 nm (Krizeket et al., 1998).

Anthocyanin measurements

To evaluate the anthocyanin content, 0.1 g of fresh tomato fruit tissue was taken and placed in a tube containing 10 ml of acidified methanol (methanol HCl, 99:1, v/v) and stored overnight at 85 °C in the dark. The homogenate was centrifuged at 4000 g for 30 minutes and the absorbance was measured at 550 nm (Wanger, 1979).

Lycopene measurements

Lycopene content was extracted using a solvent mixture of hexane, acetone, and ethanol (2:1:1,

V/V/V). Fresh tomato fruit tissue (2.5 g) was weighed, then 4 ml of distilled water was added, stirred for 60 seconds, and then it was mixed with 50 ml of the desired extraction solvent. Following that, the solution was divided into separate phases of polar and non-polar sections. A yellowcolored layer containing lycopene was removed and diluted 100 times with hexane before the sample's absorbance was measured at 502 nm (Olives Barba et al., 2006).

Ascorbic acid measurement

Titration with iodine and potassium iodide was used to measure ascorbic acid (vitamin C) (Majedi, 1994). To extract vitamin C, 10 grams of tomato pulp were homogenized for 2 minutes with a tissue homogenizer in 50 ml of the solution obtained by dissolving 30 grams of metaphosphoric acid in one liter of acetic acid. The mixture was centrifuged at 40,000 rpm at 4 $\rm{°C}$ for 15 minutes after the solution was filtered through organza fabric. The aqueous phase was put into a high-performance liquid chromatography system after filtering with a 22 um membrane. With an acetonitrile-potassium phosphate mobile phase with a flow rate of 1.5 ml/min, vitamin C was separated in a water-NH2 Bondapak column. The amount of vitamin C was calculated by estimating the area under the reaction peak using a standard vitamin C solution with a known concentration. The data were reported in mg of vitamin C per 100 g fresh fruit weight (Gonzalez-Aguilar et al., 2004).

Electrolyte leakage assay

Electrolyte leakage was calculated using a method previously described by Korkmaz et al. (2010).

Data Analysis

The SAS (Version 6.12) program was used to analyze the data. Kempthrone's line \times tester analysis was used to conduct combining ability studies (1957).

Results

Tables (1-5) show the mean of sum squares (MS) obtained for 17 physiological characteristics of tomatoes from the general analysis of variance at three levels of stress. At three levels of stress,

Table 1

Mean square from the analysis of variance for flavonoid, lycopene, ascorbic acid, and relative water content in tomato under three levels of stress

S1: control (100% FC), S2: mild stress (40% of FC), and S3: severe stress (60% of FC); ** and *: significant at P≤0.01 and P≤0.05, respectively.

Table 2

Mean square from the analysis of variance for soluble solids concentration %, pH, peroxidase, and electrolyte leakage % in tomato under three levels of stresses

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 3

Mean square from the analysis of variance for malondialdehyde, ascorbate peroxidase, and anthocyanin in tomato under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

significant differences in all variables were observed between genotypes. Similar findings were discovered for parents and crosses (Table 6- 9). Strongly significant differences in the sum squares for parents, crosses, and parent-cross interactions with all parameters at three levels of stress showed the existence of genetic differentiation among the parents and their relevant crosses, indicating that the ingredients could be used for genotypic variation progress or to adapt to selection pressure (Bhattarai, et al., 2016). This is in accordance with the improvement

Table 4

Mean square from the analysis of variance for proline, catalase, and titrable acidity %, in tomato under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 5

Mean square from the analysis of variance for chlorophyll *t*, chlorophyll *a*, and chlorophyll *b* in tomato under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 6

Estimate of general combining ability effects of parents for flavonoid, lycopene, ascorbic acid, and relative water content in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

that can be procured through the choice between several crosses, which is influenced by the degree of genetic differences among the crosses as well as the severity of the selection used. Finally, increasing the stress levels resulted in no change

in the statistically significant variances between lines, testers, and crosses in any of the traits, indicating that changing environmental conditions did not affect the differences between lines, testers, and hybrids.

Estimate of general combining ability effects of parents for soluble solids concentration %, peroxidase, and electrolyte leakage % in tomatoes under three levels of stresses.

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 8

Estimate of general combining ability effects of parents for malondialdehyde, ascorbate peroxidase, anthocyanin, and titrable acidity % in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 9

Estimate of general combining ability effects of parents for proline, catalase, and pH in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 10 Estimate of specific combining ability (SCA) effects of hybrids for agronomic characters in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 11

Estimate of specific combining ability effects of hybrids for agronomic characters in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 12

Estimate of specific combining ability effects of hybrids for malondialdehyde, ascorbate peroxidase, anthocyanin, and titrable acidity % in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 13

Estimate of specific combining ability effects of hybrids for proline, catalase, and pH in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

Table 14 Estimate of specific combining ability effects of hybrids for chlorophyl *t*, Chlorophyl *a*, and Chlorophyl *b* in tomatoes under three levels of stress

S₁: control (100% FC), S₂: mild stress (40% of FC), and S₃: severe stress (60% of FC); ** and *: Significant at P≤0.01 and P≤0.05, respectively.

General combining ability

The estimations of the effects of parents' general combining ability for all the studied traits are shown in Tables (6-9). No single line or tester was actually an excellent general combiner for each of the tested traits for the three stress levels, according to estimated lines and testers general combining ability effects, showing variances in genetic variability between parents for various traits (Arora et al., 2022; Nc et al., 2020).

The general combining ability rate in some traits such as the RWC varied with changes in stress conditions, which can be attributed to the effects of various levels of stress. As a result, no specific genotype for this trait can be proposed at all levels of stress.

Results revealed that for FLA LA2656 and LA2080, for LYC LA1579, Kingston, and Petoearly, for ASA LA1607, LA2080, LA1579, LA2656, and Kingston, for RWC Petoearly, for TSS LA1607, for PROX Petoearly, for EL LA2080, LA1579, and Petoearly, for MDA LA2656 and Petoearly, for TA LA1607 and

LA2656, for PR LA2080 and Petoearly, for CAT LA1579, for APA LA2080, LA1579 and Petoearly, for CH a LA1607, LA2656, LA1579, and Kingston, for CH b LA1607, Kingston, and Petoearly, and finally for CH T LA1607, LA1579, and Kingston were the highest general combiner since they showed significant positive GCA effects. These genotypes' positive GCA for all studied variables demonstrated that they are useful testers and lines that have indicated the dominance of their hybrids when they were either used as both or one of the parents. Also, these genotypes showed high general combining ability at all three levels, so that they can be considered in breeding programs for these traits.

Specific combing ability

Specific combining ability effects for 17 physiological attributes in 12 hybrids are shown in Tables 10-14. Results revealed that for FLA Kingston×LA1579 and Petoearly × LA2656 hybrids, for LYC Kingston × LA1579hybrid, for ASA Petoearly \times LA2656 and Kingston \times LA1579 hybrids, for RWC Bitstoik × LA2656, Kingston ×

LA1607. Kingston \times LA1579 and Petoearly \times LA2656 hybrids, for TSSKingston × LA1579 hybrid, for PROX Bitstoik × LA1607 hybrid, for EL Bitstoik × LA1607, Bitstoik × LA2080, Bitstoik × LA11579, Kingston \times LA2656, Petoearly \times LA2656 and Petoearly × LA1579 hybrids,MDA Bitstoik × LA2080, Kingston × LA1607, Kingston × LA1579, and Petoearly × LA2656 hybrids, for ANT Bitstoik × LA2656, Kingston × LA2080, Petoearly × LA2656 and Petoearly × LA1579 hybrids, for TA Bitstoik × LA2080 and Petoearly × LA1607 hybrids, for PR Bitstoik×LA2080, Kingston ×LA1607, and Petoearly × LA1579 hybrids, for CAT Bitstoik × LA2080, Kingston \times LA1607 and Petoearly \times LA1579 hybrids, for APA Bitstoik × LA1607, Kingston × LA2656, Kingston \times LA1579 and Petoearly \times LA2656 hybrids, for CH a Bitstoik × LA2080, Kingston \times LA2656 and Petoearly \times LA1607 hybrids, for CH b Bitstoik × LA2080, Bitstoik × LA1579, Kingston \times LA1607 and Petoearly \times LA2656 hybrids, and finally for CH T Bitstoik \times LA2080, Kingston \times LA1607, and Petoearly \times LA2656 hybrids showed positive SCA effects at three levels of stress while others showed negative SCA.

Gene action and variation

SCA variance had higher estimates than GCA variance, and the ratio of additive variance to nonincremental genetic variance was less than one, which indicated that non-incremental gene function was dominant in the inheritance of all traits at three stress levels. The estimates for SCA variance were higher than those for GCA variance, and the additive variance/non-additive genetic variance ratio was smaller than one, indicating that at three levels of stress, non-additive gene action predominated in the inheritance of all characteristics. On the other hand, in all traits except TSS, the degree of dominance was greater than one. These traits in tomatoes can be improved using hybrid products. Plants must be heterozygous because these properties are governed by non-additive gene action. As a result, modified breeding schemes like biparental mating, triple test mating, or the reselection generation method must be used in the early generations. In all traits and at each stress level, the ratio of additive variance to dominance

variance was estimated to be less than one (except for TSS). Results showed that, in all studied physiological traits, dominance variance was more than additive variance, and non-additive effects played a role in controlling all studied characteristics.

Of the total variations observed, i.e., the highest percentage of the line's participation at three levels of stress was observed in the FLA and ASA, but the highest percentage of tester's participation at three levels of stress was observed in MDA. Also, the highest percentage of line \times tester's participation at three levels of stress was observed in LYC, RWC, ANT, TA, PR, CAT, APA, CH a, CH b, and CH T.

Discussion

These days, there is a solid craving for crop breeding to increase yield while also improving quality. A potential approach would be to combine conventional breeding techniques with geometric profiling and interbreeding. The desire to improve nutritional characteristics (enhance flavonoid content and lycopene), extend the shelf life of tomato fruit, and improve its quality drives the conceptual strategy to improve organoleptic characteristics, particularly in tomato breeding. Many breeders have discovered through their experience with various plants that the achievements of parents alone sometimes cannot be a real index of their ability in hybrid combos. The situation for genotypes' combining ability demonstrates well how they combine with a given genome to generate survivable and productive population numbers. The breeding program can choose parents for hybridization and isolate prospective genotypes from the separate population with the support of GCA and SCA information, which also gives data on gene action that aids in understanding the principles of character inheritance (Begna, 2021).

To identify the best hybrids, estimates of SCA effects are usually used. On the other hand, studies show that SCA effects do not significantly enhance self-pollinated plants (Enang et al., 2015). According to Beyene et al. (2017), breeding plans can make use of crossovers with desirable SCA. Such programs will be more effective if one parent

is a great combiner and the other is a poor combiner. It is predicted that they will produce preferred transgressive segregants if the complementary epistatic effect and the additive genetic system in the good combiner function in the same direction to boost desirable genes of interest. The estimation of general combining ability effects aids in identifying superior parents for use in the production of superlative genotypes in separating populations through the concentration of favorable additive gene action. A strong GCA effect is also known as additive gene action or additive \times additive effects, which represent the repairable genetic components of variation (Ahamed et al., 2018). Nevertheless, at three levels of stress and with tester parents, the LA1607 showed good general combining ability for five characters, viz., ASA, TA, TSS, CH a, and CH b, at three levels of stress. The next tester was LA2656, which had a significant effect on FLA, ASA, EL, TA, and CH a. In the same way, the tester LA2080 had a significant effect on FLA, ASA, PR, APA, and EL, and finally, LA1607 had a good SCA for LYC, ASA, CAT, APA, EL, and CH a. In line parents, Petoearly, at three levels of stress, had good general combining ability for eight characters, viz., LYC, RWC, MAD, PR, APA, PROX, EL, and CH b. In this study, no hybrid exhibited superior SCA for all traits. These results agree with those for TSS and vitamin C presented by Al-Shammari and Al-Obaiday (2022) and Bharathkumar et al. (2017). Specific combing ability (SCA) variance was higher than general combing ability (GCA) variance, according to NC et al. (2020), showing that non-additive gene activity predominated for plant yields. Nonetheless, the Kingston × LA1579 mating demonstrated good specific combining ability for eight traits: FLA, LYC, ASA, RWC, TSS, MAD, and APA. Petoearly × LA2656 was the other best mating, with a significant effect on FLA, ASA, RWC, EL, MAD, ANT, APA, CH b, and CH T. In the same way, the cross Bitstoik \times LA1579 has a significant effect on EL, MAD, TA, PR, CAT, CH a, CH b, and CH T. Finally, the cross Kingston \times LA1607 showed good SCA for RWC, MAD, PR, CAT,

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