







Evaluation of agronomic traits of cotton RIL population and its parental genotypes in water deficiency environment

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Abstract

In recent years, global climate change has led to rising temperatures and droughts, which have had a negative impact on agricultural crops. Therefore, the development of new varieties of agricultural crops, including cotton, with increased resistance to abiotic stress and improved productivity remains extremely relevant using modern breeding and molecular genetic programs. In this context, it is crucial to select the initial breeding genotypes with high resistance to water stress and productivity. In this study, 100 recombinant inbred lines (RILs) of cotton (*Gossypium hirsitum* L.) from the F₁₁ population (Namangan-77 × Zangi-ota), developed by crossing the recipient cotton variety Namangan-77 with the donor variety Zangi-ota, were grown under two different conditions (water stress and optimal). Their agronomic parameters, such as seed cotton weight per boll, 1000-seed weight, fiber staple length and fiber yield, were studied. As a result of the research, it was established that 30 families of the RIL population under conditions of water deficiency showed improved indicators compared to the recipient variety Namangan-77: the seed cotton weight per boll increased by 22 %, the weight of 1000 seeds by 33 %, the fiber staple length by 4.2 % and the fiber yield by 4.4 %. Promising breeding lines from these 30 families, possessing breeding potential, were presented to the State Variety Testing Center as new drought-tolerant candidate varieties. In addition, these families, which showed high agronomic performance under drought conditions, were recommended as donors for future breeding programs and molecular genetic studies.

Keywords: agronomic traits; cotton; drought; optimal condition; recombinant inbred line

Introduction

Global climate change is leading to an increase in various abiotic and biotic stress factors. According to statistics, if such climate changes continue in this way, first, the increased demand for water will cause serious problems not only in agriculture, but also in numerous other sectors as well. This is a warning to the world about the problems we may face because of climate change.

Disparate abiotic and biotic stress factors directly affect cotton production and can significantly reduce cotton yield by 10 - 30 %. In comparison, abiotic stress factors are more severe than biotic stresses and can cause a reduction of up to 50 %. Drought stress can reduce cotton yield by up to 50 - 60 % of the total yield (1). Moreover, it is estimated that by 2050, half of current irrigated agricultural land could be affected by abiotic stressor factors (2). At high temperatures (≥36 °C), a significant decrease in carbon fixation into the air occurs, which increases the plant's susceptibility to drought stress (3 - 6). In recent years, numerous studies have shown that high temperature is one of the main abiotic factors that negatively affects cotton yield by

disrupting the photosynthesis process and membrane thermal stability, which in turn affects plant metabolism (7-9).

Drought tolerance is genetically linked to various morphological and physiological traits of plants and among abiotic stresses, drought has a significant effect on cotton growth and development, resulting in decreased cotton yield and fiber quality (10). The major reasons for the decrease in cotton yield are a reduction in the number of bolls per plant and a lower seed cotton weight per boll (11). In plants, epigenetic regulation is a critical mechanism for adaptation to stress factors. Several studies have examined the role of DNA methylation, non-coding RNA and histone modifications in plant stress responses (12). Physiological studies have shown that stomatal permeability decreases under water stress and that photosynthetic rates vary greatly among plants, reflecting potential differences in stress responses or regulatory processes such as stomatal permeability, photosynthetic rate and storage of photosynthetic products (13 - 15). Wilting of cotton plants showed that they can reduce the rate of photosynthesis under water stress. Water deficit negatively affects photosynthesis and alters the internal structure of chloroplasts, affecting chlorophyll content (16).

However, excessive drought stress can seriously damage cotton plants by disrupting the osmotic balance and cellular metabolic processes (17) and causing significant morphological changes (18). Drought stress affects plant physiological processes by affecting water balance, gas exchange and metabolic mechanisms in plants. It reduces stomatal permeability, which in turn reduces transpiration and photosynthetic efficiency, resulting in decreased chlorophyll content, carbon assimilation and energy production and negatively affects growth and yield. To overcome these effects, plants activate defense mechanisms such as accumulation of osmolytes (proline, glycine betaine and soluble sugars) to maintain cell turgor and protect membranes (19). Cotton grown under water deficit conditions has poor root development, reduced cotton yield and increased susceptibility to insect pests (20).

Drought tolerance of plants is a comprehensive indicator resulting from the interaction of various agronomic traits. Each agronomic trait is simultaneously influenced by genetic, environmental, climatic and other abiotic and biotic factors (21-23). A total of 199 genotypes were studied for six important cotton traits, such as plant height, number of bolls, weight of one boll, percentage of lint, length of the first vegetative shoot and cotton seed yield under water deficit conditions (24). As a result of numerous statistical and correlation analyses, it was found that 7 genotypes had high resistance for all traits under drought conditions and 3 had high yield under water deficit conditions.

Considering such problems, one of the urgent tasks facing scientists worldwide is to develop agricultural crop varieties that can exhibit high growth, development and high yield production in various biotic and abiotic stress environments. The aim of this study was to evaluate the resistance of cotton samples under optimal and water deficit conditions, as well as to analyze the variability of agronomic traits of genotypes under drought conditions and select resistant lines from the recombinant inbred line (RIL) population for the creation of new cotton varieties.

Materials and Methods

The object of the study was 100 recombinant inbred lines of the F_{11} cotton population (Namangan-77 × Zangi-ota), along with the cotton recipient variety Namangan-77, the donor variety Zangiota, the drought-tolerant varieties Navbakhor-2 and Ishonch as controls and the variety Toshkent-6 was used as a drought-susceptible control (25).

The study was conducted at the experimental field of the Center of Genomics and Bioinformatics, located in the Kibray district of the Tashkent region. The experimental samples were sown on the allocated plots according to the $90 \times 20 \times 1$ scheme in the third 10 days period of April. The seeds were sown to a depth of 4-5 cm, the studied materials in both conditions were placed randomly in three replicates with two rows in each replicate and 25 furrows in a row. The experimental and control variants were grown under two different irrigation regimes: optimal water supply according to the 1-2-1 scheme with a total water consumption of 4800 - 5000 m3/ha, maintaining soil moisture at a level of 65 - 70 % of the field moisture capacity during the flowering and crop setting period. The second background is a water-deficit regime, in which the total water consumption according to the limited irrigation scheme 0-1-0 is

1000-1200 m3/ha while maintaining soil moisture at a level of 40-45 % of the field moisture capacity in these same phases (26). After the plants had completed the growing season, cotton was harvested separately from each sample and parameters such as seed cotton weight per boll, 1000 - seed weight, fiber staple length and fiber yield were studied in the laboratory (27).

Statistical analyses

Analysis of variance and statistical tests of the study samples (ANOVA, Kruskal-Wallis correlation, K-Means Cluster, Tukey-Kramer) were performed using the NCSS 2017 software package. The RIL F11 (Namangan-77 × Zangi-Ota) population was analyzed for traits such as seed cotton weight per boll, 1000-seed weight, fiber staple length and fiber yield using the K-Means Cluster method. To identify drought-tolerant families of the F11 population, the Kruskal-Wallis multiple comparison Z-value test was performed. The difference between samples and confidence intervals of P-values were determined using the Tukey-Kramer test.

Results and Discussion

Many international studies have reported a decrease in the quantitative indices of morpho-biological and agronomic traits of plant species under water deficit conditions (19, 28). However, epigenomic changes in plants, including adaptation to various unfavourable stress factors and the activation of passive genes, can lead to a partial increase in these traits.

In this work, we studied the cotton F₁₁ recombinant inbred line population developed by crossing the recipient Namangan-77 and the drought-resistant donor Zangi-Ota cotton varieties (26), evaluated under both optimal and water-deficit conditions. This population consisted of 100 RIL families.

The RIL population samples were randomly planted in field plots with two irrigation regimes: water-deficit (0:1:0) and optimal (1:2:1). Each genotype were sown in 5 m rows with 3 replicates using a 90 x 20 x 1 scheme. Under water-deficit conditions various physiological processes led to a decrease in agronomical properties compared to the optimal water regime. In this study, the agronomical performance of the RIL population was evaluated under both irrigation conditions.

The raw data of the agronomic traits studied in both environments were initially organized in Excel. Then, K - Means Cluster analysis was performed on each trait of the RIL population containing 100 lines and they were divided into groups.

As a result of the analysis, a total of three distinct groups were found, which were named as follows: Group 1, consisting of 26 families, was assigned Z N RIL-min_Dr. and Z N RIL-min_Op. due to their low values. Group 2, comprising 41 families, was assigned as Z N RIL-mean_Dr. and Z N RIL-mean_Op. due to their mean values. Group 3, including 30 families, was assigned as Z N RIL-max_Dr. and Z N RIL-max_Op due to their higher values (Fig. 1).

Statistical analyses were performed on the data obtained based on K-Means Cluster analysis of morpho-biological and agronomic traits in the RIL population, their parental (recipient Namangan-77 and donor Zangi-Ota) and control (Ishonch, Navbakhor-2 and Toshkent-6) varieties using the ANOVA and general linear model (GLM) of the NCSS statistical software packages.

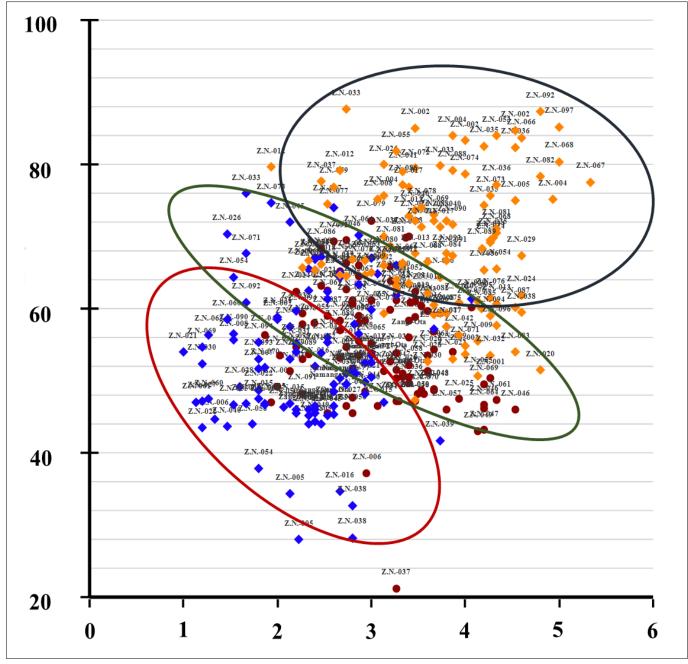


Fig. 1. K-Means Cluster analysis of agronomical parameters in RIL population, (p≤0.05).

Statistical analysis of the seed cotton weight per boll, one of the main indicators determining cotton yield, showed significant variations under water-deficit conditions. In particular, under water-deficit conditions, the seed cotton weight per boll of the cotton varieties Namangan-77_Dr, Zangi-Ota_Dr, Ishonch_Dr, Navbakhor-2_Dr and Toshkent-6_Dr was significantly lower than under optimal conditions (Fig. 2). This decrease can be explained by the fact that the samples were not provided with the required amount of water, especially during the flowering and ripening period of cotton. However, it can be observed that the seed cotton weight per boll of the variety Zangi-Ota_Dr grown under water-deficit conditions was on average 0.5 - 0.6 g higher than that of the Namangan-77_Dr variety.

In the water - deficit environment (Zangi-ota × Namangan -77 RIL), the average weight of seed cotton per boll of the Z.N.RIL-max_Dr group was 6.3 g (6.1-6.9 g), significantly higher than the drought tolerant control varieties Ishonch_Dr and Navbakhor-2_Dr (Fig. 2).

To validate the reliability of differences between the genotypes selected in the study, the Tukey-Kramer test was performed (Table 1).

The Tukey-Kramer test showed significant differences in the seed cotton weight per boll under water-deficit conditions by 10-22 % between the RILs of Z N-RIL-max_Dr. group and the varieties Namangan-77_Dr., Zangi-Ota_Dr., Z N-RIL-min_Op., Z N-RIL-min_Dr., Ishonch_Dr, Navbakhor-2_Dr, Namangan-77_Op. and Toshkent-6_Dr, with a P-value of 0.00001. In addition, the RIL values in the Z N-RIL-max_Dr. group showed differences of 9.3 % and 4.3 % compared with the Z N-RIL-mean_Dr. and Z N-RIL-mean_Op. groups, respectively, with a P value in the range of 0.001-0.0001. No significant differences in the seed cotton weight per boll were identified between the Z N-RIL-max_Dr. group and the varieties Zangi-Ota_Op., Ishonch_Op., Navbakhor-2_Op. and Toshkent-6_Op. and their P-values were \geq 0.05.

In general, according to the Tukey-Kramer test results the Z $\,$ N-RIL-max_Dr. group RILs demonstrated a superior value in

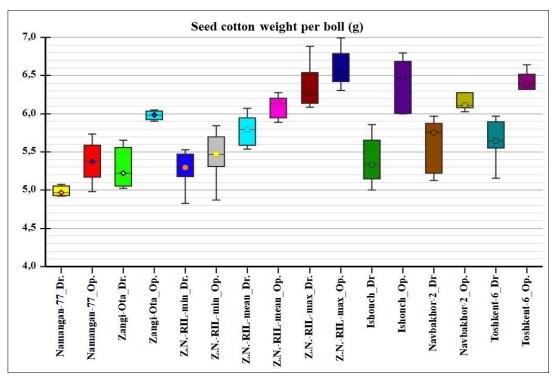


Fig. 2. Analysis of seed cotton weight per boll of the studied genotypes using the one-way analysis of variance (ANOVA) package, (p≤0.05). Dr - drought environment. Op - optimal environment.

Table 1. Results of Tukey-Kramer test (differences between all samples and confidence intervals of the P-value) for seed cotton weight per boll in the studied genotypes

in the studied genotypes								
Groups	Mean	Lower 95,	Difference Mean	Difference (%)	Upper 95,	<i>P</i> -value		
Z N-RIL-max_Dr.	6,375151							
Namangan-77_Dr.	4,983654	0,9471964	1,391497	-21,82688	1,835798	0,00001		
Namangan-77_Op.	5,373313	0,6275477	1,001837	-15,71473	1,376127	0,00001		
Zangi-Ota_Dr.	5,26776	0,7556546	1,107391	-17,37042	1,459127	0,00001		
Zangi-Ota_Op.	5,979667	-0,04881	0,395483	-6,20352365	0,8397849	0,14746		
Z N-RIL-min_Dr.	5,272872	0,8839754	1,102278	-17,29024	1,320581	0,00001		
Z N-RIL-min_Op.	5,492001	0,6732481	0,883149	-13,85300	1,093051	0,00001		
Z N-RIL-mean_Dr.	5,776403	0,3888459	0,598747	-9,391903	0,8086488	0,0001		
Z N-RIL-mean_Op.	6,09799	0,0534803	0,277161	-4,347520	0,5008419	0,00195		
Z N-RIL-max_Op.	6,607146	-0,4487048	-0,231995	3,639051	-0,01528	0,02214		
Ishonch_Dr	5,380601	0,6753539	0,994549	-15,6004148	1,313744	0,00001		
Ishonch_Op.	6,366452	-0,310497	0,008698	-0,13645167	0,3278934	1,0000		
Navbakhor-2_Dr	5,598811	0,4571441	0,776339	-12,177591	1,095535	0,00001		
Navbakhor-2_Op.	6,208119	-0,1521639	0,167031	-2,620047	0,4862265	0,91516		
Toshkent-6_Dr	5,679765	0,3761904	0,695385	-10,90775	1,014581	0,00001		
Toshkent-6_Op.	6,329249	-0,2732935	0,045901	-0,720014	0,3650969	1,00000		

terms of the seed cotton weight per boll, surpassing standard drought-tolerant cotton genotypes by up to 22 % (Table 1).

Statistical analysis of the 1000-seed weight under drought and optimal conditions revealed significant differences among the studied samples. In the Z N-RIL-max. Dr. group lines grown under water deficit conditions, the average 1000-seed weight was 132.1 g, which was not significantly different from Z N-RIL-max_Op. genotypes under optimal conditions (134.7 g), but was significantly higher than other studied samples (Fig. 3.). Under drought conditions, the 1000-seed weight of the cotton varieties Zangi-ota, Namangan-77 and Navbakhor-2 was comparatively low, namely 105 - 108 g. The Toshkent-6 variety showed the lowest value under optimal conditions - 102 g, which was below than all other samples (Fig. 3).

To validate the reliability of the results of the One-Way ANOVA statistical analysis, the Kruskal-Wallis test (multiple comparison Z-Value) was applied on the 1000-seed weight data of the studied genotypes. According to the results of the analysis, there were differences in the Z-Value value between the Z N-RIL-max_Dr. and Z N-RIL-max_Op. group lines, grown under optimal and drought conditions and all other samples (Table 2). These findings indicate that the higher-performing group was stable in terms of 1000-seed weight. Additionally, the Kruskal-Wallis correlation analysis confirmed that the Z N-RIL-max_Dr. and Z N-RIL-max_Op. groups differed significantly from the drought-tolerant varieties Ishonch and Navbakhor, the relatively susceptible.

Data analysis on the fiber staple length revealed that, the cotton varieties Zangi-Ota and Toshkent-6 under drought

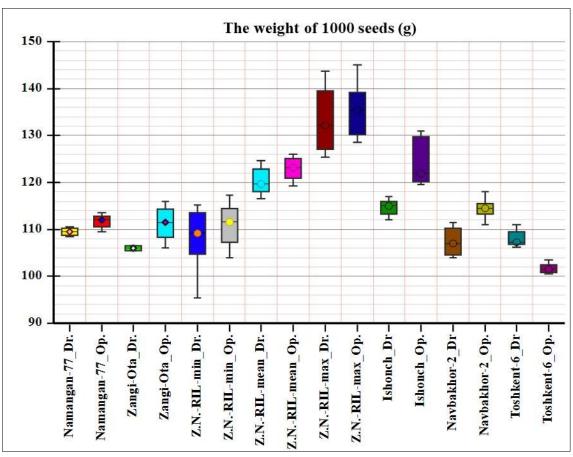


Fig. 3. Analysis of the studied genotypes in the One Way ANOVA package for the weight of 1000 seeds, ($p \le 0.05$). Dr - drought environment. Op optimal environment.

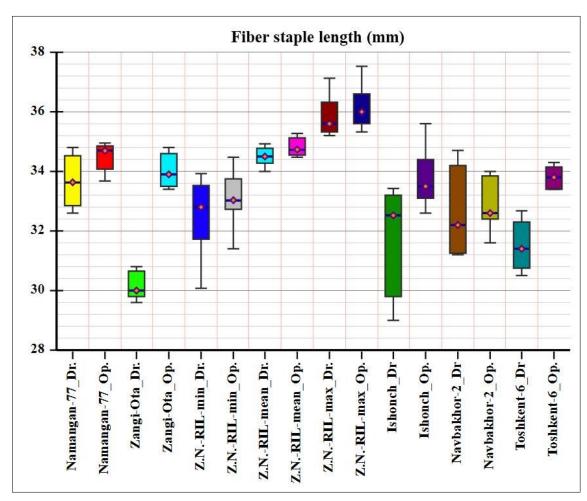


Fig. 4. One-way ANOVA analysis of variance (P≤0.05) of the fiber staple length characteristic. Dr - drought environment. Op - optimal environment.

Navbakhor Navbakhor Toshkent- Toshkent 4,838** 1,1615 7,808** 2,8769* 0,9997 5,170** 5,779** 7,517** 1,0405 4,345** 1,7155 0,1618 1,6605 3,735** 6,077** 3,6767 0,6885 0,3791 **89£'9 0,5228 4,0044** 4,3086** 0,7149 1,7153 1,0812 1,6844 2,3147* 0,0549 ,0161* 1,8223 2,0081* 6,2731** 4,5466** **6895,9 3,8385** 1,2473 1,8773 0,8253 0,5832 3,9357** 3,3781 Ishonch_Dr Ishonch_Op. 2,4188** 3,8113** 4,2583** 1,4943 1,8079 0,8064 0,1545 1,9612* 3,6573* 1,6165 1,1514 3,936** ,7613 2,2474* ,7846 4,2404* ,0572 Z.N.-RIL-3,820** 2,8143* 5,028** 9,158** 8,431** 1.215*Z.N.-RIL-8,74** 8,00** 3,381* 2,375* mean_Op. Z.N.-RIL-4,3189** 5,9812** 2,6489* 5,1785** 0,9282 2,7671* Z.N.-RILmean Dr. 2,3465* 2,2311* 3,792** 2,1507* 5,112** 4,270** 0,4004 0,0935 1,5063 0,0003 2464 0,6408 0,9443 0,5069 0,0611 Zangi-Ota_Op. 0,0674 0,3132 1,0893 0,6963 1,2131 Namangan-77 Op. 0,3887 Namangan-77 Dr. Z.N.-RIL-mean_Op. Vamangan-77_0p. Vamangan-77_Dr. Z.N.-RIL-mean_Dr. Z.N.-RIL-max_Dr. Z.N.-RIL-max_Op. Navbakhor-2_Op. Z.N.-RIL-min_Op. Z.N.-RIL-min_Dr. Navbakhor-2_Dr Zangi-Ota_Op. Foshkent-6_Dr Foshkent-6_Op Zangi-Ota_Dr. Sample name shonch_Op. shonch_Dr

Table 2. Results of Kruskal-Wallis correlation analysis on the weight of 1000 seeds of the research genotypes

*Normal test: If the Z-value is > 1.9600, the medians display significantly differences.
**Bonferroni test: If the Z-value is > 3.5931, the medians display significantly differences.

conditions displayed significantly decrease compared to their performance under optimal conditions (Fig. 4).

In the optimal environment, the fiber staple length of the Zangi-Ota and Toshkent-6 varieties was 34.0 mm and 33.8 mm, respectively, while in the drought conditions these values decreased to 30.0 mm and 31.4 mm. In contrast, under drought stress, the average fiber staple length was higher in the lines of the Z N-RIL-max_Dr. (35.8 mm) and Z N-RIL-mean_Dr. (34.6 mm) groups than in all other samples. The lowest value was found in the Zangi-Ota cotton variety (30 mm).

Within the RIL population, the Z N-RIL-min_Dr. group lines had the smallest values (32.8 mm) for fiber staple length, which were almost identical to those of the parental variety Namangan-77 (33.8 mm), the drought-tolerant varieties Ishonch (32.5 mm) and Navbakhor-2 (32.2 mm) and the drought-susceptible cotton variety Toshkent-6 (31.7 mm) (Fig. 4).

To examine the statistical reliability of the differences in fiber staple length, the Tukey-Kramer test was performed. Analysing the results, it was revealed that the Z N-RIL-max_Dr. group significantly differed from the other samples by 3.2-16.3 % ($p \ge 0.0076$ and $p \ge 0.00001$) (Table 3).

The strongest difference in the Z N-RIL-max_Dr. group under drought conditions was 16.3 % (p \geq 0.00001) and was observed in the Zangi-ota_Dr. cotton line. The smallest differences were identified in the Z N-RIL-mean_Op. group -3.2 % (p \geq 0.0076), in the Namangan-77_Op. variety -4.2 % (p \geq 0.00024) and the Z N-RIL-mean_Dr. group 4.2 % (p \geq 0.00002). Even drought-tolerant cotton varieties Ishonch and Navbakhor-2 showed lower values in both environments compared to the Z N-RIL-max_Dr. group with the differences ranged 6.2-11.7 %, p \geq 0.001 and p \geq 0.0001).

Additionally, it was found that the fiber length of the Z N-RIL-max_Dr. group differed by 6-12.5 % (p-value 0.001-0.0001) from that of the drought-susceptible Toshkent-6 variety under both optimal and stress conditions.

As is known, cotton in our republic and in many other countries is cultivated mainly for the purpose of fiber production as a raw material. One of the most important key factors determining fiber yield is the fiber yield index. The International Statistical Committee also calculates cotton yields based on fibre yield. Therefore, studying the diversity of these traits in research samples provides opportunities to develop breeding genotypes with improved fiber yield.

According to the statistical analysis of fiber yield trait, a wide variation was observed between the studied genotypes. In particular, under drought conditions, the fiber yield of the varieties Namangan-77 (35 %) and Navbakhor-2 (38.3 %) was higher compared to their values under optimal conditions (Fig. 5). The fiber yield of the variety Zangi-ota remained almost stable at 37 % under both conditions. Moreover, the Z N-RIL-max_Op. and Z N-RIL-max_Dr. groups, along with Navbakhor-2_Dr., Navbahor-2_Op., Toshkent-6_Dr. and Toshkent-6_Op. showed fiber yields of 36 - 38.5 %, like that of Zangi-Ota variety. Genotypes with fiber yields of 38 - 39 % from the Z N-RIL-max_Dr. and Z N-RIL-max_Op. groups were identified as high fiber-yielding lines. The drought-tolerant cotton variety Ishonch demonstrated lower fiber yield than Navbakhor-2, Toshkent-6, Zangi-Ota and the mean and maximum population groups. This

Table 3. Result of Tukey-Kramer test on fiber staple length (difference between all samples and confidence interval of P-value)

Sample name	Mean	Lower 95,0 %	Difference Mean	Difference (%)	Upper 95,0 %	P-Value
Z.NRIL-max_Dr.	36,008	0	0	0	0	-
Namangan-77_Dr.	33,666	0,46833	2,3422	-6,5045	4,2161	0,0016
Namangan-77_Op.	34,506	-0,1063	1,5022	-4,1718	3,1107	0,00024
Zangi-Ota_Dr.	30,137	4,34692	5,8711	-16,304	7,3954	0,00001
Zangi-Ota_Op.	34,00	0,13500	2,0088	-5,5788	3,8827	0,02174
Z N-RIL-min_Dr.	32,515	2,51754	3,4935	-9,7017	4,4694	0,001
Z N-RIL-min_Op.	32,939	2,01608	3,0691	-8,5232	4,1221	0,001
Z N-RIL-mean_Dr.	34,492	0,45053	1,5160	-4,2101	2,5815	0,00002
Z N-RIL-mean_Op.	34,830	0,15497	1,1783	-3,2723	2,2016	0,0076
Z N-RIL-max_Op.	36,268	-1,3072	-0,2599	0,7218	0,7874	0,99997
Ishonch_Dr	31,771	2,83331	4,2373	-11,767	5,6414	0,0001
Ishonch_Op.	33,744	0,86040	2,2644	-6,2885	3,6684	0,001
Navbakhor-2_Dr	32,642	1,96269	3,3667	-9,3497	4,7707	0,0001
Navbakhor-2_Op.	32,966	1,63818	3,0422	-8,4485	4,4462	0,0001
Toshkent-6_Dr	31,507	3,09733	4,5013	-12,500	5,9054	0,0001
Toshkent-6_Op.	33,788	0,81595	2,22	-6,1651	3,6240	0,001

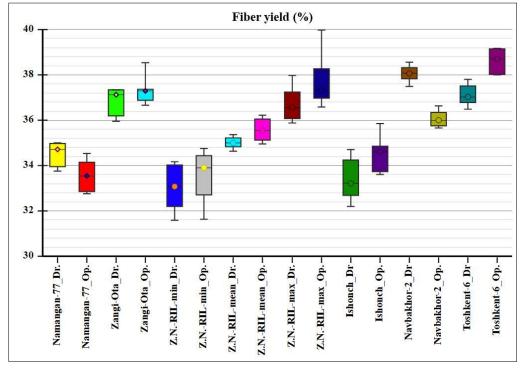


Fig. 5. One-way ANOVA analysis of variance (P≤0.05) of fiber yield data for the research samples. Dr - drought environment. Op - optimal environment.

decrease is likely due to its higher 1000-seed weight than other varieties, resulting in lower fibre yield.

Conclusion

In this study, agronomical parameters, including the seed cotton weight per boll, 1000-seed weight, fiber staple length and fiber yield of the F11 (Namangan-77 × Zangi-Ota) RIL population, were evaluated under water-deficit and optimal conditions compared with the recipient variety Namangan-77, the donor variety, Zangi-Ota and the controls, the drought-tolerant varieties Navbakhor-2 and Ishonch and drought-susceptible variety Toshkent-6. It was found that 30 families of the F11 (Namangan-77 × Zangi-Ota) RIL population (Z N-RIL-max group) displayed improvements under water deficit conditions in comparison to the recipient Namangan-77: the seed cotton weight per boll by 22 %, the 1000-seed weight by 33 %, 4.2 % in fiber staple length and 4.4 % in fiber yield.

From these 30 RIL families, promising genotypes with improved agronomical traits were individually selected and

submitted for registration to the State Variety Testing Center as new drought-tolerant candidate varieties.

In addition, these families with superior agronomic performance under drought conditions were recommended as donors for future breeding and molecular genetic studies.

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Authors' contributions

NNK conducted the laboratory and field experiments, collected the data, performed statistical analysis and wrote the manuscript. MMK, JKN, ISN, UAB, AKM, RIM, SIM, ABM, YAM, IEB

and BKR participated in the experiments and revision of the manuscript. ZTB revised and approved the final manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

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