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SALINITY, *VERTICILLIUM* WILT TOLERANCE AND GENETIC DIVERSITY ANALYSIS OF UPLAND COTTON GENOTYPES

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Abstract. Cotton is one of the most economically important crops. Screening and selection salt-tolerant cotton genotypes using DNA molecular markers not only introduces tolerant cultivars valuable for hybridization and breeding programs, but also identifies DNA regions implicated in the salinity tolerance mechanism. Screening and selection of cotton genotypes resistant to salinity stress and Verticillium wilt disease using DNA molecular markers not only introduces tolerant cultivars valuable for hybridization and breeding programs, but also identifies DNA regions implicated in the tolerance mechanism. The lowest Disease Severity Index was recorded in AP-317 (0.37) and Tashkent-2 (0.32) varieties. A total of 63 bands were generated based on 12 ISSR primers, 50 of which were polymorphic. The highest PIC value was recorded for UBC841 (0.466). AP-317 of Azerbaijan origin and Kirqizistan-174 of Kyrgyzstan origin were the most distant (0.358) genotypes and at the same time showed high resistance to *Verticillium* wilt. The obtained results showed that ISSR markers were an effective tool for determining genetic variation and identification of cotton cultivars.

Keywords: *Gossypium hirsutum, Verticillium wilt, salinity, genetic diversity, polymorphism*

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1. Introduction

Upland cotton (*G. hirsutum*) is the most important fiber crop for the global textile industry (Akparov *et al*., 2021; Mammadova *et al*., 2021; Alizada, 2022; Zhang *et al*., 2023). As a globally important cash crop, cotton provides approximately 35% of total fiber used worldwide and is also an important source of oilseed (Cui *et al*., 2021). Cotton is grown in more than 80 countries and plays an important role in social and economic development of Azerbaijan (Alizada *et al*., 2024a).

There are about 20 major diseases affecting cotton including *Verticillium* wilt, which is a vascular disease that is caused by soil-borne fungus Verticillium dahlia (ElSharawy *et al*., 2015). The fungus invades through the roots and once in the xylem

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produces conidiospores that spread throughout the plant. During infection, the *V. dahliae* secretome supplies a range of molecules to manipulate the host responses and aid its growth that can result in vascular occlusion, which prevents the transfer of water and other mineral substances from roots to the leaves and tissues and causes wilting, drying, a reduction in photosynthesis, shedding of immature bolls and importantly a significant reduction in fiber yield. In the field, the disease is characteristically associated with vascular discoloration, leaf chlorosis, necrosis and plant death in severe cases (Wilson, 2024). The disease would occur in the whole growth period of cotton and reach the peak in the fowering and boll-setting period from July to August and the infected cotton leaves would gradually turn yellow, wither and fall of, which would lead to small cotton bolls and high boll drop rate and fnally result in a decrease in yield and quality (Lu *et al*., 2023).

Strategies opted so far to control V. dahliae infection are not enough. Research at morphophysiological, biochemical and molecular levels is required to understand the infection mechanism and most importantly the response to cotton towards infection is critical (Umer *et al*., 2023). Because of its highly variable pathogenicity and strong vitality, it is extremely difficult to control *Verticillium* wilt disease (Zhang *et al*., 2018).

Salt stress is the second most prevalent abiotic stress after drought, impairing plant growth and reducing agricultural production worldwide. Due to poor management practices and lack of regulation, salt stress is getting worse every year. Saline irrigation increases the amount of sodium chloride in the soil, which can lead to soil degradation (Anwar *et al*., 2023).

Cotton yield decreases at a salinity level of 7.7 dS m^{-1} and a 50% reduction in output was noted at 17.0 dS m^{-1}. Cotton plants are affected in multiple ways by salt stress, including diminished growth, limited leaf area expansion and impaired nutrient uptake (Alizada, 2024b). Under salt stress, fiber strength, length and micronaire values decrease in both Gossypium hirsutum and *Gossypium barbadense*. Moreover, the early developmental stages of cotton are particularly vulnerable to salt stress, which has a significant impact on eventual crop output (Anwar *et al*., 2023; Alizada *et al*., 2020).

Salt tolerance is a quantitative trait which is affected by the environmental factors. However, selection based on genetic rather than phenotypic characteristics is a fast, reliable and cost effective approach which can enhance the identification of tolerant cotton genotypes. Inter-simple sequence repeat (ISSR) fingerprinting is a PCR based method was developed such that no prior sequence knowledge was required (Ahmadizadeh *et al*., 2011; Khayatnezhad *et al*., 2010; Zaefizadeh *et al*., 2009). This technique has proven to be a simple, quick and inexpensive method which can generate high percentages of polymorphic loci. ISSR technique reported as an easy and informative genetic marker system for revealing both inter and intraspecific variations in cotton, yielding a multi locus marker system useful for fingerprinting, diversity analysis and genome mapping (Abdi *et al*., 2012).

This study aimed to determine the of *Verticillium* wilt resistant genotypes, also investigation genetic diversity of 31 cotton cultivars that studied under salt stress conditions, compare genetic distance estimated from ISSR markers.

2. Materials and methods

Plant material

Seeds of 31 cotton (*G. hirsutum* L.) varieties were obtained from National Genbank of Azerbaijan. Information about varieties is presented in Table 1.

Table 1. List of studied genotypes

Genotype	GenBank ID	Origin	Genotype	GenBank ID	Origin
Aghdas-3	AzGR-10139	Azerbaijan	Navai-9	AzGR-3591	Uzbekistan
AP-317	AzGR-3601	Azerbaijan	Kirqizistan-174	AzGR-3590	Kyrgyzstan
Barakat	AzGR-11836	Azerbaijan	Beyaz altun-440	AzGR-13638	Turkey
Bayraqdar	AzGR-10202	Azerbaijan	Carisma	AzGR-13640	Turkey
Ganja-110	AzGR-5852	Azerbaijan	CSN-12	-	Turkey
Ganja-114	AzGR-7733	Azerbaijan	Edessa	AzGR-13637	Turkey
Ganja-160	-	Azerbaijan	Flash	AzGR-13639	Turkey
Ganja-182	AzGR-11468	Azerbaijan	Lima	AzGR-13636	Turkey
Ganja-195	AzGR-12215	Azerbaijan	May-344	-	Turkey
Ganja-200	AzGR-12216	Azerbaijan	PG	AzGR-13641	Turkey
Kharabakh-11	AzGR-835	Azerbaijan	Sezener-76	-	Turkey
Kharabakh-12	-	Azerbaijan	Assos	-	Greece
Zafar	AzGR-11839	Azerbaijan	Prime	-	Greece
Tashkent-1	AzGR-5396	Uzbekistan	Select	-	Greece
Tashkent-2	-	Uzbekistan	Cristina	-	Greece
Tashkent-3	-	Uzbekistan			

To evaluate *Verticillium* wilt resistance, plants were planted in 3 replicates and 10 plants per replicate in 2019-2021 at the Absheron Research Base of the Institute of Genetic Resources on an artificial wilt background. At the end of the vegetation period, the evaluation of the disease according to the cross-section of the stem was performed according to the Karman (1971) method. The disease severity index (DSI) was calculated using the following formula:

$$DSI = \frac{0a + 1b + 2c + 3d + 4e}{n}$$

DSI: Disease severity index; a, b, c, d, e:
The plant number with degree 0, 1, 2, 3, 4 respectively,
n = total number of plants

The 0-4 scale used in determining the reaction against *Verticillium* Wilt:

0 - No visible chlorosis on the leaf; **1 -** chlorosis in 1/4 of the leaf; **2 -** 1/2 chlorosis, necrosis or paleness on the leaf; **3 -** chlorosis or necrosis in 2/3 of the leaf; **4 -** The leaf is ready to fall or has fallen;

DNA extraction and amplification

For molecular-genetic analysis fresh true leaf samples of 31 cotton varieties were collected, frozen in liquid nitrogen and stored at - 80˚ until the DNA extraction. Total DNA extraction was performed using CTAB method with modifications (Doyle and Doyle, 1990). DNA quantity and purity determined by using Nanodrop 2000c (ThermoFisher Scientific, USA, Cat. No. / ND2000CLAPTOP). 15 primers were used in the study and 12 primers were polymorphic. Information about polymorphic primers is presented in Table 2.

Table 2. List of polymorph primers and primer sequence

Primer	Sequence 5`-3`
ISSR-1	AGACAGACGC
ISSR-2	GACAGACAGACAGACA
ISSR-3	AGAGAGAGAGAGAGAGC
ISSR-7	CACACACACACACAGT
ISSR-12	GTGTGTGTGTGTGTTG
ISSR-14	GTGTGTGTGTGTGTCT
ISSR-16	CACACACACACAAG
ISSR-19	AGAGAGAGAGAGAGAGT
UBC816	CACACACACACACACAT
UBC823	TCTCTCTCTCTCTCTCC
UBC825	ACACACACACACACACT
UBC841	GAGAGAGAGAGAGAGAYC

Polymerase Chain Reactions were carried out by using FIREPol® Master Mix (Solis Biodyne, Estonia, Cat. No. / 04-11-00125) in a 25 µl volume mixture containing as follows: 5 µl of a total DNA, 4 µl primer (5 µM), 2 µl dNTPs (200 mM), 2.5 µl Taq buffer (10X), 2 µl $MgCl_2$ (25 mM) and 1.5 U of DNA polymerase. The samples were amplified on a Biobase Digital Thermal Cycler (Bioer Technology, China, Cat. No. / TC-96/G/H(b)C). PCR program was: 1 cycle hot start, 94°C (3 min); 35 cycles, 94°C (1 min), (45-55)°C (1 min), 72 (2 min); 1 cycle extension, 72°C (7 min) and then cooling down to 4°C. The amplified ISSR products were separated separated and analysed using in 1.8 % agarose gel with 0.5X TBE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8) containing 0.5 µg/ml ethidium bromide and visualized under Chemidoc XRS system (Bio-Rad, USA, Cat. /No. / 721BR07370). The absence or presence of fragments was scored as 0 or 1 for each band, in order to determine variation between accessions.

Marker's data analysis

Data analysis was done by SPSS v25 software package. Cluster analysis was generated using Ward's method based on Euclidean distances was conducted. PopGene 1.32 software was used to calculate Shannon`s Information Index.

The genetic diversity index was determined using the formula proposed by Weir (1990):

$$H = 1 - \Sigma p^2_i$$

where H – index of genetic diversity, pi – frequency of alleles.

The PIC value for each locus was calculated according to the formula of Roldan-Ruiz et al. (2000):

$$PIC_i = 2fi\ (1 - fi),$$

where PICi is PIC for locus i, fi is the frequency of amplified fragments and $1 - fi$ is the frequency of nonamplified fragments.

For the calculation of RP, information of a band (BI) was measured as:

$$BI = 1 - (2x\ |0.5\text{-}p|),$$

where p is the percentage of the six species having bands (Raza *et al*., 2020).

Then, RP of each primer was measured as:

$$RP = \sum_{i=1}^{n} BI_{\mathrm{i}},$$

where n is the total number of bands for that marker (Prevost & Wilkinson, 1999).

MRP for each marker calculated as:

$$MRP = 1/n \sum_{i} BI_{\mathrm{i}}.$$

EMR was measured as a total number of polymorphic bands/per primer multiplied by the percentage of the polymorphic bands per their total number.

$$EMR = np\ \left(\frac{np}{n}\right),$$

where *np* is the number of polymorphic bands and n is the total bands number. The high EMR value indicates the more efficiency of the primer-marker system (Raza *et al*., 2020). MI is a statistical parameter used to calculate the total effectiveness of the primer-maker system. MI is the product of the PIC values and EMR (Raza *et al*., 2020). MI was measured as:

MI = PIC x EMR.

3. Results and discussion

Table 3. DSI and salinity tolerance of studied genotypes

Genotype	DSI	Salt tolerance	Genotype	DSI	Salt tolerance
Aghdas-3	0.79±0.05	Moderate	Navai-9	0.58±0.06	High
AP-317	0.37±0.03	High	Kirqizistan-174	0.50±0.04	High
Barakat	0.44±0.05	Moderate	Beyaz altun-440	0.55±0.08	High
Bayraqdar	0.58±0.04	Moderate	Carisma	1.07±0.12	Sensitive
Ganja-110	0.95±0.01	Moderate	CSN-12	1.22±0.14	Moderate
Ganja-114	1.02±0.11	Moderate	Edessa	1.49±0.21	Moderate
Ganja-160	0.47±0.05	Moderate	Flash	0.62±0.07	Moderate
Ganja-182	0.50±0.05	Sensitive	Lima	0.46±0.05	Moderate
Ganja-195	0.49±0.03	Moderate	May-344	0.79±0.06	Moderate
Ganja-200	0.43±0.05	Moderate	PG	0.95±0.11	Moderate
Kharabakh-11	0.80±0.07	Moderate	Sezener-76	0.73±0.08	Moderate
Kharabakh-12	1.29±0.03	Moderate	Assos	0.60±0.02	Moderate
Zafar	0.46±0.05	Moderate	Prime	0.42±0.05	Moderate
Tashkent-1	1.07±0.17	Moderate	Select	0.52±0.03	Moderate
Tashkent-2	0.32±0.03	High	Cristina	0.91±0.07	Moderate
Tashkent-3	0.45±0.04	High			

Among the studied Azerbaijani cotton varieties, AP-317 received the lowest value according to the DSI indicator and was highly resistant to wilt disease, while Kharabakh-12 genotype was evaluated as highly sensitive (Table 3). Furthermore, the salinity (NaCl) resistance of these varieties were evaluated based on biometric parameters in our previous studies (Alizade, 2022; Alizade *et al*., 2023c) and the AP-317 variety was evaluated as a highly resistant variety to salt stress (NaCl) in our previous studies (Alizade *et al*., 2023a; 2023b). Carisma variety from Turkey that sensitive to salt stress and CSN-12, Edessa varieties, which were evaluated as moderately resistant to salt stress, were evaluated as sensitive genotypes to *Verticillium* wilt. Among the introduced varieties, the highly salt-resistant Tashkent-2 and Tashkent-3 varieties were observed to have a low rate of infection with wilt disease.

Polymorphism detected by ISSR analysis:

The selection an appropriate molecular marker technique for measuring genetic diversity requires careful evaluation of criteria such as statistical power, reliability and the number of polymorphisms (Mammadova *et al*., 2023; 2024).

Table 4. Marker parameters calculated for each ISSR primer

Primer	**Band size (bp)**	**TNB/ TNPB**	**PR, %**	***PIC***	***H***	**EMR**	**RP**	**MI**	**MRP**	**I**
ISSR-1	200-800	3/3	100.0	0.448	0.853	3.00	2.13	1.34	0.71	0.472
ISSR-2	250-850	3/3	100.0	0.423	0.801	3.00	1.87	1.27	0.62	0.452
ISSR-3	350-950	4/4	100.0	0.387	0.751	4.00	2.19	1.55	0.55	0.547
ISSR-7	400-900	9/8	88.9	0.349	0.853	7.11	2.9	2.48	0.36	0.489
ISSR-12	350-500	7/5	71.4	0.455	0.793	3.57	2.06	1.63	0.41	0.527
ISSR-14	550-950	6/4	66.7	0.396	0.737	2.67	2.39	1.06	0.60	0.47
ISSR-16	450-900	4/4	100.0	0.436	0.801	4.00	2.84	1.74	0.71	0.638
ISSR-19	350-950	8/5	62.5	0.46	0.876	3.13	3.48	1.44	0.70	0.478
UBC816	450-950	5/4	80.0	0.453	0.851	3.20	3.35	1.45	0.84	0.498
UBC823	350-1200	6/5	83.3	0.394	0.783	4.17	2.58	1.64	0.52	0.571
UBC825	450-1100	4/3	75.0	0.437	0.812	2.25	1.65	0.98	0.55	0.459
UBC841	350-1000	4/2	50.0	0.466	0.716	1.00	2.45	0.47	1.23	0.536
Total		**63/50**								
Mean		**5.25/ 4.17**	**81.48**	**0.425**	**0.802**	**3.42**	**2.49**	**1.42**	**0.65**	**0.511**

TNB-Total number of amplified bands; **TNPB**-Total number of polymorphic bands; **PR**-Polymorphism rate; **PIC**-Polymorphism Information Content; **H**-Genetic diversity index; **EMR**- Effective multiplex ratio; **RP**- Resolving power; **MI**-**M**arker index; **MRP**- **Mean** resolving power; **I**-Shannon information index

12 polymorphic primers were used for the study of genetic diversity and the characteristics of the primers are presented in Table 4. A total of 63 bands were synthesized on these primers, 50 of them were polymorphic. The total number of bands varied between 3-9 and the total number of polymorphic bands varied between 2-8. ISSR-1, ISSR-2, ISSR-2 and ISSR-16 had the highest polymorphism at 100% and UBC841 had the lowest at 50%. The highest value of PIC was observed in UBC841 (0.466) and the lowest value was observed in ISSR-7 (0.349). Genetic diversity (H) index varied between 0.716 (UBC841) and 0.876 (ISSR-19). Bilwal et al. (2017) studied genetic diversity of 9 cotton genotypes and obtained 209 total band for 22 ISSR primers of which 142 were polymorphic. Sagar et al. (2020) during his research on 9 elite cotton genotypes, among 15 polymorphic ISSR primers observed a total of 460 alleles with an average of 11.80

bands per primers. They also noted the maximum PIC value (0.68) for primer ISSR 32 and UBC 842. Sahin et al. (2018) observed average PIC value 0.41, with minimum PIC 0.19 and maximum PIC 0.68 during the analysis conducted with 24 ISSR primers on 30 cotton varieties.

Table 5. Genetic similarity index of studied cotton varieties

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1-Ganja-114	1,000																														
2-Cristina	0,500	1,000																													
3-Sezener-76	0,625	0,760	1,000																												
4-Barakat	0,519	0,551	0,536	1,000																											
5-Ganja-195	0,545	0,547	0,533	0,633	1,000																										
6-Tashkent-1	0,596	0,600	0,661	0,623	0,736	1,000																									
7-Zafar	0,558	0,529	0,517	0,520	0,783	0,630	1,000																								
8-Navai-9	0,563	0,532	0,547	0,556	0,462	0,414	0,412	1,000																							
9-Kirqizistan-174	0,563	0,565	0,577	0,458	0,382	0,414	0,358	0,737	1,000																						
10-Aghdash-3	0,714	0,688	0,655	0,612	0,604	0,685	0,528	0,531	0,531	1,000																					
11-Tashkent-2	0,661	0,552	0,667	0,630	0,621	0,786	0,579	0,527	0,474	0,691	1,000																				
12-Tashkent-3	0,625	0,571	0,633	0,623	0,643	0,782	0,630	0,491	0,464	0,685	0,923	1,000																			
13-Ganja-110	0,647	0,620	0,542	0,519	0,667	0,655	0,653	0,442	0,471	0,714	0,632	0,685	1,000																		
14-Ganja-200	0,620	0,472	0,571	0,462	0,519	0,544	0,529	0,532	0,532	0,528	0,579	0,571	0,588	1,000																	
15-Carisma	0,648	0,654	0,745	0,500	0,525	0,627	0,483	0,569	0,633	0,618	0,633	0,655	0,534	0,564	1,000																
16-Edessa	0,582	0,647	0,709	0,519	0,492	0,649	0,474	0,472	0,592	0,611	0,627	0,649	0,526	0,500	0,878	1,000															
17-Kharabakh-11	0,673	0,647	0,649	0,519	0,660	0,593	0,585	0,472	0,500	0,740	0,600	0,593	0,673	0,474	0,673	0,667	1,000														
18-PG	0,561	0,536	0,627	0,556	0,667	0,714	0,593	0,509	0,481	0,618	0,750	0,778	0,618	0,654	0,679	0,586	0,586	1,000													
19-CSN-12	0,556	0,588	0,655	0,580	0,574	0,655	0,558	0,471	0,500	0,615	0,632	0,685	0,556	0,688	0,712	0,673	0,582	0,854	1,000												
20-Ganja-160	0,596	0,630	0,633	0,536	0,643	0,690	0,600	0,464	0,491	0,717	0,667	0,690	0,784	0,467	0,600	0,621	0,774	0,655	0,596	1,000											
21-Kharabakh-12	0,527	0,620	0,569	0,580	0,574	0,492	0,473	0,500	0,596	0,647	0,550	0,569	0,615	0,528	0,589	0,582	0,740	0,589	0,615	0,655	1,000										
22-AP-317	0,519	0,551	0,483	0,574	0,702	0,564	0,727	0,489	0,400	0,549	0,571	0,593	0,646	0,462	0,448	0,464	0,608	0,615	0,580	0,623	0,580	1,000									
23-Assos	0,547	0,612	0,534	0,674	0,627	0,648	0,646	0,553	0,431	0,608	0,685	0,679	0,808	0,411	0,526	0,466	0,545	0,673	0,577	0,648	0,519	0,711	1,000								
24-Ganja-182	0,787	0,588	0,885	0,612	0,574	0,596	0,653	0,563	0,531	0,647	0,661	0,685	0,647	0,620	0,589	0,526	0,582	0,589	0,585	0,569	0,585	0,580	0,808	1,000							
25-May-344	0,698	0,611	0,796	0,441	0,517	0,617	0,450	0,558	0,588	0,636	0,623	0,590	0,552	0,582	0,792	0,722	0,691	0,610	0,607	0,617	0,607	0,417	0,467	0,636	1,000						
26-Select	0,564	0,729	0,722	0,473	0,500	0,603	0,456	0,510	0,604	0,623	0,610	0,632	0,564	0,566	0,896	0,894	0,648	0,625	0,686	0,603	0,623	0,473	0,500	0,536	0,736	1,000					
27-Lima	0,588	0,529	0,600	0,551	0,547	0,630	0,500	0,532	0,469	0,653	0,636	0,660	0,558	0,625	0,593	0,556	0,556	0,720	0,761	0,571	0,571	0,558	0,583	0,580	0,620	0,611	0,537	1,000			
28-Prime	0,542	0,692	0,690	0,536	0,643	0,661	0,571	0,547	0,577	0,596	0,613	0,633	0,625	0,467	0,745	0,649	0,649	0,694	0,596	0,719	0,625	0,536	0,648	0,596	0,672	0,661	0,492	1,000			
29-Flash	0,467	0,604	0,610	0,482	0,648	0,667	0,574	0,386	0,463	0,517	0,617	0,667	0,630	0,466	0,722	0,717	0,655	0,632	0,600	0,696	0,630	0,537	0,536	0,492	0,621	0,731	0,518	0,696	1,000		
30-Beyaz altun-440	0,571	0,574	0,667	0,509	0,561	0,610	0,545	0,519	0,549	0,600	0,644	0,667	0,544	0,635	0,661	0,655	0,655	0,755	0,796	0,696	0,660	0,537	0,564	0,571	0,679	0,636	0,771	0,583	0,614	1,000	
31	0,731	0,642	0,702	0,604	0,655	0,702	0,582	0,500	0,473	0,800	0,707	0,732	0,698	0,582	0,667	0,603	0,722	0,667	0,667	0,764	0,636	0,518	0,630	0,698	0,684	0,614	0,673	0,672	0,621	0,709	1,000

Cluster analysis of 31 cotton cultivars was used to produce a dendrogram showing the genetic relationships between genotypes based on ISSR data and Jaccard's similarity coefficient (Figure 1). The varieties clustered in the 1st clade originated from Azerbaijan and Uzbekistan. Tashkent-2 and Tashkent-3 genotypes of Uzbekistan origin were highly resistant varieties due to *Verticillium* wilt and salt resistance. The local varieties clustered in this clade mainly showed moderate resistance to salt stress.

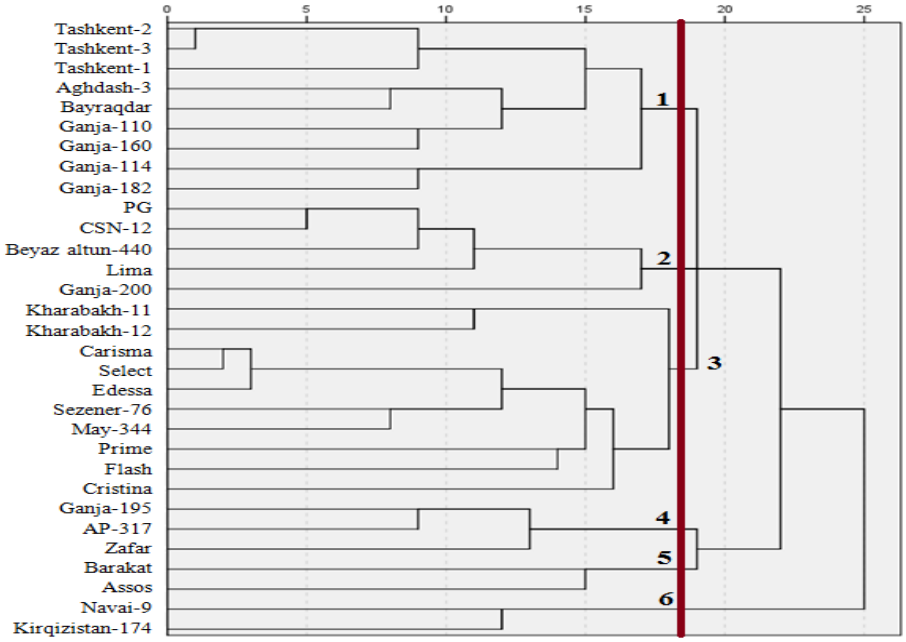

Figure 1. Dendrogram showing genetic diversity among selected cotton genotypes

The varieties concentrated in the 2nd clade were mostly Turkey varieties and in this group Beyaz altun - 440 variety was highly resistant to salt stress, while other genotypes were moderately resistant. The 3rd clade was the largest group and the cultivars concentrated in this group mainly showed moderate resistance to both stress factors. Varieties clustered in 4th group were local varieties and were selected high resistance to *Verticillium* wilt. Both cultivars clustered in clade 5 showed moderate resistance to salt stress. The cultivars concentrated in clade 6 were genotypes highly resistant to salt stress. Varieties included in this group are also distinguished by their high resistance to *Verticillium* wilt.

4. Conclusion

Genetic recombination from natural diversity is abundant and can be used to test for salt tolerance. One useful tool that can be used in this type of investigation is DNA molecular markers.

In this study the maximum PIC values were found for primers UBC 841 and ISSR 12, indicating that these primers made the most contribution to the diversity analysis. Furthermore, the results indicated that the ISSR molecular marker could be a valuable tool in cotton breeding for salt tolerance; however, further research using additional primers could explore this possibility.

The tree cluster analysis resulted that AP-317 and Kirqizistan-174 genotypes were quite distinct. These genotypes from various clusters can be used in hybridization to ensure the production of heterotic combinations for breeding programs. The current study's result on genetic relatedness using pooled molecular makers data will be useful for selecting parents for crossbreeding, expanding the genetic bases of breeding materials and improving cotton breeding.

References

Abdi, A.A., Sofalian, O., Asghari, A., Shokrpour, M., Baqheri, H. & Masoumi S.Y.S. (2012). Inter-Simple Sequence Repeat (ISSR) markers to study genetic diversity among cotton cultivars in associated with salt tolerance *Notulae Scientia Biologicae*, *4*(4), 120-126.

Ahmadizadeh, M., Shahbazi, H., Valizadeh, M. & Zaefizadeh, M. (2011). Genetic diversity of durum wheat landraces using multivariate analysis under normal irrigation and drought stress conditions. *African Journal of Agricultural Research*, *6*(10), 2294-2302.

Akparov, Z.I., Mamedova, R.B., Guseynova, L.A., Abdulalieva, G.S., Yunusova, F.M. & Alizade, Sh.A. (2021). Competitive evaluation of perspective cotton lines in variety development nursery. *Advances in Current Natural Sciences*, 10, 7-12.

Alizada Sh., Aliyeva K. (2024a). Comparative analysis of expression profiles of antiporter encoding gene (*GhNHX1*) under different concentrations of NaCl in cotton (*Gossypium hirsutum* L.). *Advances in Biology & Earth Sciences*, *9*(*1*), 168-174. https://doi.org/10.62476/abes9168

Alizada, S. (2022). Role of miRNAs in cotton salt stress responses. *Advances in Biology & Earth Sciences, 7*(1), 80-84.

Alizada, S., Guliev, R., Mammadova, R. & Zaefizadeh, M. (2020). System perspective analysis for molecular and genetic source of salt tolerance in cotton. *Khazar Journal of Science and Technology, 4*(1), 70-83.

Alizade, S. (2024b). Comparative evaluation of *GhCIPK6* gene expression profiles under different concentration of NaCl in cotton (*G. hirsutum* L.) seedlings. Bulletin of Perm University. Biology, 1. (In Russian).

Alizade, S., Mammadova, R. & Sirajli, N. (2023b). Evaluation of morphometric traits of upland cotton genotypes under different concentration of NaCl. *Advances in Biology & Earth Sciences*, *8*(3), 301-307.

Alizade, Sh. (2022). Comparative study of SPAD values in cotton plant under salt stress. *Proceedings of Genetic Resources Institute of ANAS, 11*(1), 139-146.

Alizade, Sh., Mammadova, R. (2023a). Assessment of salt stress resistance of cotton varieties based on different parameters. *Advances in Biology & Earth Sciences*, *8*(*1*), 58-66.

Alizade, Sh.A. (2023c). Evaluation of the *GhMAPK* gene expression level under salt stress in cotton cultivars. *Plant Biotechnology and Breeding, 6*(4), 40-47. (In Russian).

Anwar, Z., Ijaz, A., Ditta, A., Wang, B., Liu, F., Khan, S.M.U.D., Haidar, S., Hassan, H.M., Khan, M.K.R. (2023). Genomic Dynamics and Functional Insights under Salt Stress in *Gossypium hirsutum* L. *Genes, 14*, 1-25.

Bilwal, B.B., Vadodariya, K.V., Rajkumar, B.K., Upadhyay, A.K. & Lahane, G.R. (2017). Genetic diversity of parents using RAPD, ISSR and SSR molecular markers in upland cotton (*Gossypium hirsutum* L.). *Bulletin of Environment, Pharmacology and Life Sciences*, *6*(8), 51-57.

Cui, Y., Ge, Q., Zhao, P., Chen, W., Sang, X., Zhao, Y. & Wang, H. (2021). Rapid mining of candidate genes for verticillium wilt resistance in cotton based on BSA-seq analysis. *Frontiers in Plant Science*, 12, 703011.

Doyle, J.J., Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 11-15.

ElSharawy, A.A., Yang, G., Hu, X. & Yang, J. (2015). Genetic relationships between virulence, vegetative compatibility and ISSR marker of Verticillium dahliae isolated from

cotton. *Archives of Phytopathology and Plant Protection*, *48*(8), 646-663. http://dx.doi.org/10.1080/03235408.2015.1091164

Kahodariya, J., Sabara, P. & Vakharia, D. (2015). Assessment of genetic diversity in old world and new world cotton cultivars using RAPD and ISSR markers. *Indian Journal of Biotechnology*, 14, 511-517.

Karman, M. (1971). General information in plant protection investigations, evaluation and performing of the trials. *Regional Agricultural Protection Institute*, 279.

Khayatnezhad, M., Zaefizadeh, M., Gholamin, R. & Jamaati-e-Somarin, S. (2010). Study of genetic diversity and path analysis for yield in durum wheat genotypes under water and dry conditions. *World Applied Sciences Journal*, *9*(6), 655-665.

Lu, Z., Huang, S., Zhang, X., Shi, Y., Yang, W., Zhu, L. & Huang, C. (2023). Intelligent identifcation on cotton verticillium wilt based on spectral and image feature fusion. *Plant Methods*, 19, 1-17. https://doi.org/10.1186/s13007-023-01056-4

Mammadova, R., Akparov, Z., Amri, A., Bakhsh, A., Alo, F., Alizade, S., Amrahov, N. & Yunisova, F. (2024). Genetic diversity analysis of Azerbaijani bread wheat (Triticum aestivum L.) genotypes with simple sequence repeat markers linked to drought tolerance. *Genetic Resources and Crop Evolution*, 1-9.

Mammadova, R., Amri, A., Akparov, Z., Alo, F., Sheikhzamanova, F., Abbasov, M., Amrahov, N., Hajiyev, E. & Alizade, S. (2023). Evaluation of genetic diversity using SSR markers and link with drought response of Azerbaijani durum wheat (Triticum durum Desf.) genotypes. *Genetic Resources and Crop Evolution*, 1-10.

Mammadova, R., Huseynova, L., Abdulaliyeva, G., Yunusova, F. & Alizade, S. (2021). Prospects of the remote hybridization on improvement of the main economical traits of cotton genotypes with naturally colored fibre. *East European Scientific Journal*, *6*(70), 4-8.

Prevost, A, Wilkinson, M. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics*, 98, 107–112. https://doi.org/10.1007/s001220051046

Raza, A., Farooq, A.B.U., Khan, W.A., Iqbal, A., Çelik, S., Ali, M. & Khan, R.S.A. (2020). Polymorphic information and genetic diversity in Brassica species revealed by RAPD markers, *BIOCELL*, *44*(4), 769-776.

Roldan-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A. & De Loose, M.A.F.L.P. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.). *Molecular Breeding*, 6, 125-134.

Sagar, Nimbal, S., Jangid, K. & Kumar, P. (2020). Molecular diversity analysis among elite genotypes of American cotton (*Gossypium hirsutum* L.). *Pantnagar Journal of Research*, *18*(1), 1-4.

Shahin, C.B., Ishler, N. & Rustamova, V. (2018). Characterization of some cotton varieties using ISSR markers. *International Eurasian Conference on Biological and Chemical Sciences*, 553.

Sofalian, O., Azimy, S., Jahanbakhsh, S., Khomari, S. & Dezhsetan, S. (2013). Evaluating genetic diversity of chilling stress in cotton genotypes. *Plant Breeding and Seed Science*, 68, 77-87.

Umer, M.J., Zheng, J., Yang, M., Batool, R., Abro, A.A., Hou, Y. & Zhang, B. (2023). Insights to Gossypium defense response against Verticillium dahliae: The Cotton Cancer. *Functional & Integrative Genomics*, *23*(2), 142. https://doi.org/10.1007/s10142-023-01065-5

Weir, B.S. (1990). *Genetic Data Analysis Methods for Discrete Genetic Data*. Sunderland, USA. Sinauer Assoc. Inc.

Wilson, I.W., Moncuquet, P., Yuan, Y., Soliveres, M., Li, Z., Stiller, W. & Zhu, Q. H. (2024). Genetic mapping and characterization of verticillium wilt resistance in a recombinant inbred population of upland cotton. *International Journal of Molecular Sciences*, *25*(4), 2439.

Zaefizadeh, M., Goliev, R. (2009). Diversity and relationships among durum wheat landraces (subconvars) by SRAP and phenotypic marker polymorphism. *Research Journal of Biological Sciences*, *4*(8), 960-966.

Zhang, J., Zhu, Y., Wheeler, T. & Dever, J.K. (2023). Development and validation of allele-specific PCR-based SNP typing in a gene on chromosome D03 conferring resistance to Fusarium wilt race 4 in Upland cotton (Gossypium hirsutum). *Molecular Genetics and Genomics*, *298*(6), 1579-1589. https://doi.org/10.1007/s00438-023-02079-1

Zhang, Q., Gao, X., Ren, Y., Ding, X., Qiu, J., Li, N. & Chu, Z. (2018). Improvement of Verticillium wilt resistance by applying arbuscular mycorrhizal fungi to a cotton variety with high symbiotic efficiency under field conditions. *International Journal of Molecular Sciences*, *19*(1), 241.