MINISTRY OF AGRICULTURE AND WATER RESOURCES OF UZBEKISTAN

SCIENTIFIC RESEARCH INSTITUTE OF SELECTION, SEED-GROWING AND AGRO-TECHNOLOGY OF COTTON PLANT CULTIVATION

EFFICIENCY OF SELECTING ON THE BASIS OF MOLECULAR MARKERS FOR THE PURPOSE OF G.HIRSUTUM L.COTTON GRADES CREATION

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Recently more and more attention is given to application of molecular markers in selection processes (marker assisted selection) for increase of selection processes efficiency of necessary genotypes of plants. Molecular markers are used widely by selectors of the USA, the European Union, Australia, Japan, China, Russia and other countries. According to - Dubcovsky (2004), the program «MAS wheat» operating in the USA, in 2004 covered about 430 projects directed on transfer of 43 genes in 75 parental forms of wheat.

Therefore introduction of molecular markers in the traditional - scheme of selection demands an implementation of preliminary researches on their efficiency studying at selection and development of individual technique for the concrete selection program. On the basis of this method we defined genotypic stability to natural virulent populations of Verticillium fungi, and also their homogeneity on morpho-biological signs and presence of markers on early maturation.

However, it is necessary to notice that molecular markers should possess certain properties and to meet certain requirements:

- High level of polymorphism;
- Co-dominant character of inheritance;
- Occurrence of optimum level in genome for specific tasks solution;
- Uniform distribution in genome on chromosomes;
- Selectively neutral behaviour;
- Easy estimation of markers parameters;
- Possibility of automation of markers parameters estimation;
- High reproducibility of markers parameters estimation.

Any fragments of DNA used for revealing of polymorphism can serve as molecular markers. In practice the experimenter, as a rule, deals not with a gene-marker, he deals with its phenotypic display – well expressed, discrete, i.e. a qualitative sign. The latter can be considered as the factor of identification of a gene corresponding to it, i.e. as a gene marker, and also the genes linked to it. The reliable marker is a protein sign, as protein is a primary product of a gene (V.G.Konarev, 1974, 1983; A.A.Sozinov, 1982; S.Yunuskhanov, A.Ibragimov, 1988; R.Abidov, 2001).

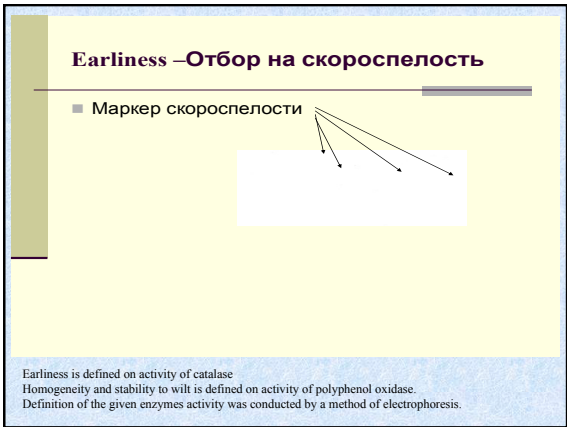
In this connection the researches on fastening of transgressive forms of plants with a complex of useful signs (earliness, stability to more virulent populations of verticilliose and vascular wilt, productivity, homogeneity, a high output and quality of a fiber, stability to adverse conditions of environment) on the basis of various methods of crossing (repeated, sating, stepped, complex) and selection methods (pedigree, mass, periodic, stabilizing) have been conducted, and also on the basis of molecular markers to define degree of genetic homogeneity on morpho-biological signs at families on earliness and wilt-resistance.

As initial material

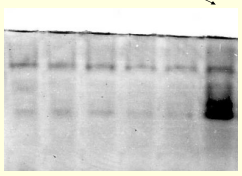
For experience conducting served the families, lines and complex back-cross hybrids $F_{4,}$ bred in biological nursery (30 families), the 1-st year selection nursery (20 lines) and the 2-nd years selection nursery (13 families).

Experimental procedure.

Selection of adaptive genotypes was conducted on technology developed by Professor R.Shadmanov in laboratory of applied biochemistry of plants of the Institute of Genetics and Experimental Plant Biology.

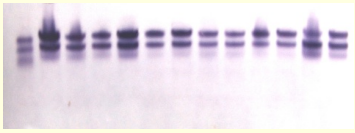


Tolerance to Wilt – Отбор на вилтоустойчивость



Marker on wilt resistance

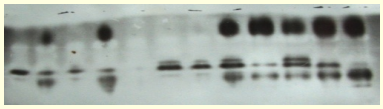
Variety improved on purity
Сорт после выравнивания



Marker on homogeneity

Evaluation of varietal purity

Оценка сортовой чистоты

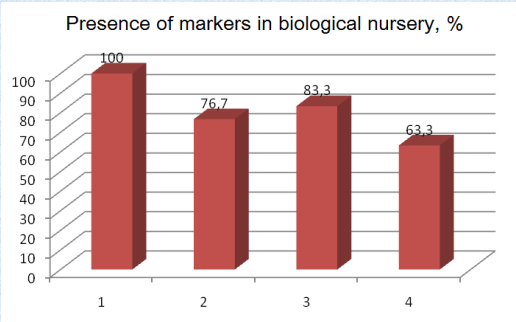


Сорт до чистки

Heterogeneous forms

The conducted researches have shown that in biological nursery from 30 families studied, 23 families had the markers on earliness that makes 76,7 %, on wilt-resistance 25 families (83,3 %) and on homogeneity on morpho-biological signs - 18 families (60 %) (Diagram). In 1-st year selection nursery from 20 studied lines the markers on earliness and wilt-resistance had all lines, and on homogeneity 14 (70 %) (Tab. 2). Had no markers on homogeneity such lines as L-651, L-1726, L-1875, L-2146, L-2161, L-2268. In 2-nd years selection nursery from 13 lines a marker on homogeneity had 11 lines, i.e. 84,6 %, and at lines L-1642 and L-335 the above-stated marker was not revealed (Tab. 3).

Comparative studying of families and lines on homogeneity in biological nursery has shown that the period from shoots appearance prior to the beginning of the first bolls opening at all families lasted 106-118 days whereas at grade C-6524 it made 119 days, i.e. families had earliness for 1-13 days. The family 145 (106 days) was the earliest, and the late-ripening were 4 families (693, 1140, 1299 and 37) (Table 1).



1 – Total of the studied families;
2 – Markers on earliness;
3 – Markers on wilt-resistance;
4 – Markers on homogeneity.

Table 1

The basic economic-valuable signs and their markers
(The biological nursery of complex hybrids F_4 natural wilt background)

№	Family and line	Grades and hybrid combinations	Earliness, days	Wilt infection, %, As of 10.09		Output of fibre, %	Length of fibre, mm	Largeness of bolls,
				In total	Including strong degree			
		C-6524 (St)	119	89,4	68,5	35,2	33,5	4,9
1.	356	F_3 [F_1B_1 (F_1 Omad x LS-485) x	115	8,1	-	36,3	33,0	5,7
2.	431	L-030] x (F_1B_1 (F_1 C-6530 x	114	8,6	-	40,4	34,6	5,5
3.	463	LS-485) x L-030]	113	5,3	-	36,9	34,4	6,0
4.	594	F_3 [F_1B_1 (F_1 Omad LS-489) x	113	6,0	2,2	39,7	34,8	6,0
5.	598	L-030] x (F_1B_1 (F_1 C-6530 x LS-489) x L-030	115	10,0	-	35,3	34,0	5,8
6.	668	[F_1B_1 (F_1F_3 Omad LS-6592) x	114	9,0	-	38,3	34,0	5,4
7.	693	L-030] x [F_1B_1 (F_1 C-6530 x LS-6592) x L-030]	118	10,3	-	39,3	35,0	5,6
8.	723	F_3 [F_1B_1 (F_1 Omad x LS-6593) x	114	12,7	-	36,6	34,4	6,2
9.	799	L-030] x [F_1B_1 (F_1 C-6530 x LS-6593) x L-030	113	8,3	-	39,5	33,0	5,7
10.	893		114	16,5	0,8	38,7	34,4	6,2

11.	919		110	15,8	-	38,6	34,0	6,0
12.	931	F_3 [F_1B_1 (F_1 Omad x LS-6594) x	113	7,5	1,0	40,8	34,6	6,0
13.	1029	L-030] x [F_1B_1 (F_1 C-6530 x LS-6594) x L-030]	116	11,0	-	40,2	36,8	6,1
14.	1030	F_3 [F_1B_1 (F_1 Omad x LS-6595) x	116	6,5	-	38,3	35,8	6,0
15.	1098	L-030] x [F_1B_1 (F_1 C-6530 x	117	10,8	-	40,2	33,4	5,9
16.	1140	LS-6595) x L-030]	118	7,5	4,9	43,3	34,0	6,0
17.	1286	F_3 [F_1B_1 (F_1 Omad x LS-6596) x	113	5,5		36,9	34,4	6,0
18.	1299	L-030] x [F_1B_1 (F_1 C-6530 x LS-6596) x L-030]	118	-	-	37,8	34,2	5,6
19.	1396	F_3 [F_1B_1 (F_1 Omad x LS-6598) x	109	-	-	36,7	35,4	5,5
20.	1402	L-030] x [F_1B_1 (F_1 C-6530 x LS-6598) x L-030]	112	-	-	37,3	35,0	5,9
21.	1443	F_3 [F_1B_1 (F_1 C-8284 x LS-484) x	111	-	-	37,0	33,2	6,2
22.	37	L-030 [F_1B_1 (F_1 Omad x LS-484] x L-030	118	-	-	38,9	34,6	6,0
23.	21		109	-	-	40,8	35,0	6,1
24.	29	F_3 [F_1B_1 (F_1 C-8284 x LS-485] x L-030] x [F_1B_1 (F_1 Omad x LS-485) x L-030	112	-	-	39,2	34,8	6,2
25.	128		110	17,1	-	37,6	34,0	6,0
26.	145		106	16,0	-	38,0	34,8	5,8
27.	209		111	17,6	-	39,4	33,0	5,9
28.	339		108	16,5	-	41,3	33,0	5,6
29.	355		110	18,3	-	37,9	35,6	5,4
30.	363		109	11,0	-	39,4	35,2	5,8

In 1-st year selection nursery all studied lines exceeded on earliness the standard for 5-20 days at which this period lasted from 98 till 113 days whereas at grade C-6524 it was equal to 118 days.

The line L-651 has the shortest vegetative period (98 days), and at other lines it makes 107-113 days, i.e. these lines earlier than grade C-6524 for 5-9 days. It means that we managed on the basis of marker tests to create early lines of cotton (Table 2).

In 2-nd years selection nursery, the earlier created lines on homogeneity of morpho-economic signs, earliness and wilt-resistance were studied.

From resulted Table 3 it is visible that the created lines had shorter vegetative period from shooting till the first bolls opening, than the standard grade C-6524 for 2-13 days.

The greatest earliness possessed the lines L-1384, L-1222, L-1460, L-83 and L-932 at which the vegetative period makes 105-109 days. At other lines it is equal to 110-116 days, but, despite it they were earlier than the standard for 2-8 days.

Table 2.

The basic economic-valuable signs and their markers
(The selection nursery of the 1-st year, a natural wilt background).

№	Lines	Markers			Earliness, days	Wilt infection, %, As of 10.09		Output of a fibre, %	Length of a fibre, mm	Largeness of bolls, gr
		On earliness	On wilt	On homogeneity		In total	Including strong degrees			
	C-6524 (St)	+	-	+	118	85,2	67,8	34,7	34,0	5,3
1.	L-651	+	+	-	98	11,3	-	38,3	33,4	6,3
2.	L-1698	+	+	+	112	10,8	-	37,0	33,6	7,0
3.	L-1726	+	+	-	110	18,4	-	38,5	33,2	6,5
4.	L-1753	+	+	+	113	19,8	-	36,5	33,4	6,8
5.	L-1762	+	+	+	110	15,5	-	36,0	34,2	6,4
6.	L-1875	+	+	-	108	14,4	-	36,1	33,6	6,7
7.	L-1823	+	+	+	109	16,2	-	36,9	34,6	6,0
8.	L-1928	+	+	+	110	17,3	-	36,5	34,0	6,9
9.	L-1959	+	+	+	107	15,5	-	36,9	34,4	6,6
10.	L-2004	+	+	+	108	16,8	-	37,8	34,2	6,1
11.	L-2074	+	+	+	111	18,4	-	38,7	35,0	6,2
12.	L-2146	+	+	-	110	15,4	-	39,5	35,4	6,2
13.	L-2161	+	+	-	112	17,8	-	40.0	35,2	6,3
14.	L-2291	+	+	+	113	18,5	-	38,4	35,8	6,8
15.	L-2303	+	+	+	112	19,3	-	43,6	34,6	6,7
16.	L-2235	+	+	+	110	13,0	-	37,4	35,4	6,2
17.	L-2268	+	+	-	113	18,3	-	36,4	36,8	5,6
18.	L-2108	+	+	+	109	16,1	-	38,2	34,8	6,2
19.	L-2156	+	+	+	112	13,5	-	39,5	35,4	6,2
20.	L-2195	+	+	+	110	10,7	-	40.0	35,2	6,3

Table 3.

The basic economic-valuable signs and their markers
(The selection nursery of the 2-nd year, a natural wilt background).

№	Lines	Markers			Earliness, days	Wilt infection, %, As of 10.09		Exit of a fibre, %	Length of a fibre, mm	Largeness of bolls, gr
		On earliness	On wilt	On homogeneity		In total	Including strong degrees			
	C-6524 (St)	+	-	+	118	78,9	73,5	35,0	33,8	5,0
1.	L-1309	+	+	+	116	12,8	-	37,3	35,4	6,7
2.	L-1335	+	+	+	114	16,6	-	37,8	34,4	7,4
3.	L-1384	+	+	+	105	17,0	-	37,1	33,6	6,2
4.	L-1642	+	+	-	112	14,6	-	38,4	34,8	6,3
5.	L-1285	+	+	+	108	13,2	-	38,8	33,4	7,3
6.	L-1222	+	+	+	109	19,7	-	38,3	35,2	6,0
7.	L-1203	+	+	+	112	16,8	-	36,0	34,0	6,9
8.	L-335	+	+	-	112	18,8	-	37,0	34,0	5,6
9.	L-1306	+	+	+	115	14,6	-	39,0	35,2	5,8
10.	L-1460	+	+	+	107	10,0	-	40,6	34,0	6,0
11.	L-559	+	+	+	112	10,8	-	38,8	34,2	5,8
12.	L-83	+	+	+	109	17,9	-	42,6	34,1	6,9
13.	L-932	+	+	+	108	11,2	-	38,5	34,4	6,5

Wilt infection in all studied nurseries was much lower at families and lines, in comparison with a standard grade. So in biological nursery C-6524 grade was infected by wilt in the general degree on 89,4 % and in strong degree on 68,5 %, and from 30 families only 23 families in the general degree on 5,3-18,3 %, and 4 families in strong degree (594, 893, 931 and 1140) on 1,0-4,9 % (Tab. 1) were infected.

Wilt infection was also much lower in comparison with a standard grade and in the 1-st year selection nursery. In particular, if grade C-6524 was diseased in the general degree on 85,2 %, at lines this indicator has made from 10,7 % to 19,8 %. It means that created by us lines exceed much more a standard grade on wilt-resistance. In strong degree, unlike a standard grade (67,8 %), no one of the lines was infected by the given disease (Tab. 2). In drawings 1, 2 and 3 the presence of markers on homogeneity and wilt-resistance of standard grade C-6524 and lines L-1753 and L-2268 is shown.

In the 2-nd years selection nursery, the indicators on wilt-resistance were identical to the indicators received in the previous nurseries. So, grade C-6524 was infected by wilt in the general degree on 78,9 % and strong degree on 73,5 % whereas at lines this sign varied within 10,0-19,7 % (Tab. 3). The most wilt-resistant line was L-1460 (10,0 %), and the most unsteady - L-1222 (19,7 %). In drawing 4 the presence of markers both on homogeneity and on wilt-resistance at lines L-335 and L-1460 is shown.

It is necessary to notice that at the created lines, the biochemical markers on earliness, wilt-resistance and homogeneity of morpho-biological signs specifying that the studied lines have the genes supervising these signs (fig. 1-4) are revealed.

Natural wilt background, grade C-6524

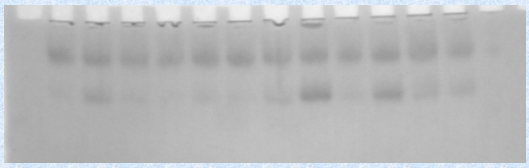


Fig. 1. A *standard grade C - 6524, strongly infected by wilt*

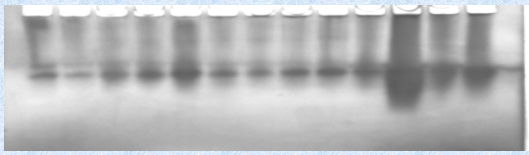


Fig. 2. *L-1753 line is homogeneous, but partially infected by wilt*

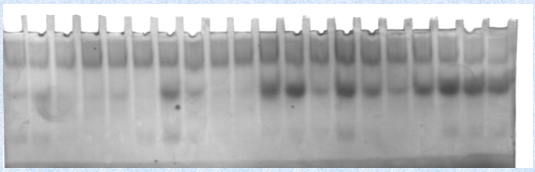


Fig. 3. *L-2268 line is non- homogeneous and partially infected by wilt*

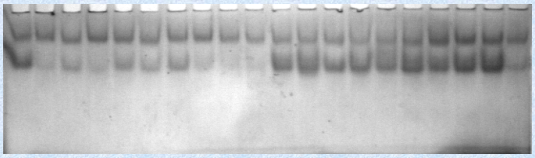


Fig. 4. From *1 to 10 – line L-335* is *non-homogeneous and infected by wilt;*
From *11 to 20 - line L-1993 is homogeneous and steady to wilt infection*

In the course of lines selecting on the basis of molecular markers have been allocated highly productive, early maturing, wilt-resistant and genotypic rather homogeneous lines of cotton (L-1309, L-1335, L-1384, L-1642, L-1255, L-1222, L-1203, L-335, L-1306, L-1460, L-559, L-83, L-932) to be breed for the purpose of their transfer to competitive test.

At the same time we spent inoculation of a plant-owner by new virulent isolates of Verticillium dahliae Kleb. fungi for definition of genotypic stability of new created cotton lines. Inoculation of a plant-owner spent with virulent isolates which have been allocated from diseased plants (collected in autumn) by laying of diseased pieces into the damp chamber, and then fungi's re-sowing in subers on Czapek's medium or by a direct premise of pieces of the infected plants on Czapek's medium to test tubes, passing the damp chamber. Inoculation of plants spent the employees of Scientific Research Institute of Plants Protection under the direction of Professor A.Marupov.

The phenotypic account of diseased plants by August, 20th. From resuls of Table 4 it is visible that at two studied lines in reproduction nursery at inoculation of a plant-owner by new virulent isolates of Verticillium fungi the phenotypic display of wilt has been revealed. The quantity of diseased plants varied from 16,6 % to 66,4 %, depending on genotypic stability of new lines. The highest phenotypic wilt-resistance displayed the lines L-2674, L-2324, L-2107, L-1749, L-1435 and L-573 at which the quantity of diseased plants has made from 16,6 to 33,3 %.

The genotypic analysis of wilt-resistance at studied lines in reproduction nursery at inoculation of a plant-owner by new virulent isolates of Verticillium fungi on a cut of the main stalk has shown that the lines studied do not possess absolute immunity to new isolate, but had a various degree of the main stalk's infection. So, for example, from Table 4 it is visible that new lines, basically, had weak and medium degree of the main stalk's infection. It varied from 0 % to 66,4 % in weak degree and in medium from 0 % to 49,8 %. In strong degree it is observed at 4 lines: L-2107, L-45, L-573 and L-1211 which degree of the main stalk's infection makes from 16,6 % to 33,2 %. It means that new lines possess relative immunity to new virulent isolates of Verticillium fungi.

Table 4.
Stability of new cotton lines at inoculation of a plant-owner by new virulent pathogen of Verticillium dahliae Kleb. fungi (Reproduction nursery)

№	Lines	Wilt dynamics, %			In total	On a cut of a stalk, %, 10.09			In total	Distinction between a phenotype and a cut of a stalk, %
		June 28.06	July 07.07	August 20.08		weak	medium	strong		
	C-6524 (St)	33,2	49,9	100,0	100,0	16,6	49,8	33,2	94,7	-
1	L-2674	0	33,2	33,2	33,2	33,2	33,2	0	66,4	33,2
2	L-2627	0	0	49,9	49,9	49,9	0	0	49,9	0
3	L-2366	16,0	0	49,8	49,8	49,8	16,6	0	66,4	16,6
4	L-2324	16,6	0	16,6	16,6	16,6	16,6	0	33,2	16,6
5	L-2107	0	16,6	33,2	33,2	16,6	16,6	16,6	49,8	16,6
6	L-1993	0	0	49,8	49,8	49,8	0	0	49,8	0
7	L-1749	16,6	0	16,6	16,6	16,6	16,6	0	33,2	16,6
8	L-1435	0	33,2	33,2	33,2	33,2	33,2	0	66,4	33,2
9	L-45	16,6	33,2	33,2	33,2	33,2	33,2	16,6	83,0	49,8
10	L-573	16,6	16,6	33,2	33,2	0	33,2	33,2	66,4	33,2
11	L-115	0	16,6	49,8	49,8	33,2	33,2	0	66,4	16,6
12	L-374	0	0	49,8	49,8	33,2	16,6	0	49,8	0
13	L-1211	0	0	66,4	66,4	33,2	16,6	16,6	66,4	0
14	L-2473	16,6	0	49,8	49,8	33,2	33,2	0	66,4	16,6

Conclusions.

Summarizing the results of studying of new families and the lines of cotton received at different systems of crossings on presence of markers on earliness, wilt-resistance and homogeneity of morpho-economic signs it is possible to notice that the selection work should be spent on the basis of biochemical markers as it allows to define genetic leveling of a selection material on earlier generations, thereby to reduce volume of selection work and to reduce terms of deducing of new perspective families, lines and the grades of cotton possessing high earliness, wilt-resistance with a complex of economic-valuable signs.

1. On homogeneity the complex back-cross hybrid families F_4B_1 are characterized by heterozygosis. From 30 studied families only at 17 families the marker on homogeneity of morpho-economic signs is revealed, whereas at other families they are absent. It means that genotypic homogeneity of families on the basis of a biochemical marker makes 56,7 %. Whereas, the other families on phenotypic display of morpho-economic signs are rather homogeneous.

2. The selector at phenotypic selection of hybrid plants F_4B_1 on a complex of morpho-economic signs in most cases selects the heterozygous plants. Therefore with a view of more effective selection work it is necessary to apply biochemical markers for estimation of a selection material on stability of signs in early generations.

3. In selection nurseries of the 1-st and 2-nd years all lines have markers on earliness and wilt-resistance. On homogeneity the markers are not found out in 6 lines in selection nursery of the 1-st year and at 2 lines in selection nursery of the 2-nd year.

4. Wilt-resistance in all studied nurseries on natural wilt background was much more above, than at standard grade C-6524. Grade C-6524 was infected by wilt in the general degree on 78,9-89,4, and in strong degree on 67,9-73,5, whereas at the studied families and lines prevalence of disease in the general degree has made from 0 to 18,3 %.

5. The new highly productive, homogeneous, early maturing, wilt-resistant lines created were studied in reproduction nursery, for the purpose of definition of phenotypic stability of lines to new virulent isolates of Verticillium fungi at its inoculation into the organism of a plant-owner. The type of wilt-resistance depends on reaction of a genotype of new lines and is characterized by super sensitivity and tolerant stability to studied isolate.

- A high wilt-resistance to new virulent isolates of Verticillium fungi possess the lines L-2627, L-2324, L-1207, L-1993, L-1749 and L-374.

Thank you for your attention!