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Original scientific paper

INVESTIGATION OF SUBLINES OF KRAGUJEVAČKA 56 VARIETY OF WHEAT (TRITICUM AESTIVUM SSP. VULGARE)

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Chosen sublines of the Kg. 56 cultivar were examined with main goals of detecting polymorphism within it and of identifying superior genotypes for some traits. Some morphological, physiological and technological traits were investigated, as well as resistance to disease (in general, traits with high heritability) using standard methods. Polymorphism of sublines was detected within the Kg. 56 cultivar and confirmed on a biochemical basis, by polyacrylamide gel electrophoresis of grain endosperm gliadins. Using the acid PAGE–method 4 different genotypes were identified in relation to gliadin composition, and on the basis of the remaining analyses 11 sublines were identified.

INTRODUCTION

Variability within bean (Phaseolus sp.) cultivars was noted by Johannsen (1903, 1909, 1923) and he defined the term 'pure line'. Variability within barley and oat cultivars, which had originated by individual selection, was noted by Allard (1960) and Hayes *et al.* (1955). Miladinović and Žikić (1969) observed the existence of four stable genotypes within Bezostaja – 1

cultivar. Within some Yugoslav wheat cultivars Javornik (1989) and Vapa (1989) found the existence of different genotypes by biochemical analyses. Allard produced the Fulghum oats cultivar, which was created from a single plant by individual selection and which was reselected again to pure line cultivars such as Kanota, Frazier and Franklin. Boerma and Cooper (1975) found that pure lines of soybean could be isolated from superior heterogenous lines, which could be the same or even better, than heterogenous lines.

In addition to backward hetorozygosity, mixture and cross – pollination, an important source of variability could be mutations. According to East (1936) mutations were noticed at a high rate for undefective genes. Mayo (1987) wrote similarly about mutations.

The final result of autogamy is a homozigous but not homogenous population. In autogamous species, plant of the F–5 generation, which were selected by the pedigree method, were hetorozygous for one or more locuses. That is especially the case when several locuses were segregated. In the case of the existance of selective differences between homozygotes and heterozygotes (at the loss of former) homozygosity appeared much more slowly than theoretically anticipated. Small coefficients of selection at neutral mutations influence the rate of mutation which is an important factor in polymorphisms (Falconer, 1981). The reflection of variation is conditioned by different genotypic in different behavior environments (Haldane and Jayakar, 1963), etc.

Borojević (1981) intimated that a pure line cultivar should consist of more than 95% of the same genotype. He deffined a pure cultivar as a set of very similar genotypes with the sample phenotype. Furthermore, he stated that it is possible to select pure cultivar from isolated lines of the F_4 generation by the pedigree method. Bradshaw (1965) noted that stability of yield is conected with an environment – cultivar reaction, *i.e.* with phenotype plasticity conditioned by cultivar genetic composition. Borojević (1981) stated that pure line adaptability is specific and corresponds only for a specific agroecological area. Adaptability and yield stability of a pure cultivar is higher than in a pure line, so a pure cultivar usually occupies a larger growing area.

In order to explain variability within the Kragujevačka 56 cultivar, some investigations of the most frequent sublines of this cultivar were made.

MATERIAL AND METHODS

Kg. 56 cultivar was created by crossing (Bezostaja – 1 x Halle stamm) x Bezostaja–1. Cultivar Kg. 56 was released in 1975 by the Yugoslav Federal Commission for Cultivar Approval. It was selected by the pedigree method to the F_4 generation and from the F_5 and further generations by progeny selection, (Popović, 1984). Variability in quantitative traits was noticed within the Kg. 56 cultivar. That indicated the existence of some genotypes with similar phenotypes. Mostly those genotypes belong to *Tr. aestivum* ssp. *vulgare* var. *lutescens*. Exceptionaly genotypes with red or awned spikes were noticed. They probably arose by mutations of genes conditioning these qualitative traits. Backward hetorozygosity, according to Borojević (1981), is a very important cause of variability in cultivars created in way that Kg. 56 has been created. This

heterozygosity had been stabilized in later generations in the form of many pure lines. Considerable differences in quantitative traits between parent cultivars can also contribute to variability which is the case in Kg. 56. These differences make homozygosity slower to be achieved.

At the time of Kg. 56. cultivar selection, this variability was accepted as desirable because of increasing adaptability. After approval of the Kg. 56 cultivar this variability was maintained. During maintance by individual selection only extreme types were discarded.

The eleven most frequent genotypes of this cultivar were investigated in this work from 1984 to 1989. Genotypes were sown on the experimental plots (5 x 5 m^2) by the randomised complete block system. Traits with high heritability were investigated, such as: height of plant, date of heading, date of ripeness, time of kernel filling, sedimentation value, 1000 kernel weight and hectolitre mass (B o r o j e v i ć, 1986, D e P a c e *et al.*, 1978, etc.). Analyses were made in the laboaratories of the Institute for small grains – Kragujevac by standard methods. Data were treated to variance analysis. The cold resistance was tested in frozen chambers by the method of Jurijev. Resistance to different races of *Puccinia graminis tritici*, was tested in greenhouse and field conditions. Lodging was assessed during 5 years, the resistance to low temperature during two years and other traits were assessed during 4 years. The results of these analyses were obtained by counting, measuring and visual assessment. Hues of red color were assessed visualy, from 1 (lightest) to 6 (darkest).

Analyses of storage proteins (gliadins) in grain for these sublines were made by the improved method of L o o k h a r t (1982) in the Grain Marketing Research Laboratory, Manhattan, K.S., USA.

RESULTS

The results of analyses of morphological an physiological traits for 11 sublines and the Kg. 56 cultivar as a standard are shown in Table 1. The appearance of some lines in the milk maturity phase is presented in Figures 1a and 1b. It is possible to see the existence of differences for most of the investigated traits. During the investigation all lines showed uniformity and stability.

From emergence time to heading time was 163 days for line Kg. 56/17, 166.3 days for Kg. 56/20 and for Kg. 56/27, (Tab. 2). Line Kg. 56/36 had the shortest time from heading to ripening but Kg. 56/20 had the longest. Lines showed significant differences from standard Kg. 56, only for time of kernel filling.

The traits which were investigated had high levels of heritability. When the lines were compared significant differences between the traits were found.

Reisstance to diseases was investigated in greenhouse and field conditions (Fusarium sp. and Septoria sp.). The results are shown in Table 3. Difference between lines were evident. The most resistance lines were Kg. 56/4 and Kg. 56/17.

Some parameters of grain quality also with a high level of heritability were investigated (Table 4). Significant differences between lines for 1000 kernel weight were noted. The highest mass was found for Kg. 56/17 and the lowest for

Table 1. Morphological and phisiological traits of sublines of Kragujevačka – 56 cultivar of wheat

Line – – cultivar	Date of heading 1985/86. –1988/89.	Date of ripening 1985/86. –1988/89.	Height of plant with spike (cm) mean (± St.) 85/86–88/89.	Lodging resistance (0–9) '84–85–'88/89.	Resistance to low temperatures (–14°C 24h) Mean for '87/88–'88/89.	Shade of grains red color (1–6)	Other observations
KG–56/4	7.V– 2.VI	30.VI –13.VII	85,3(–2.3)	0,00	96.25	3	Almost erectile top leaf, more ashen layers
KG–56/9	10.V– 2.VI	1.VII–13.VII	84,5(–2,9)	0.13	100.0	2	Top leaf of flag type
KG–56/13	10.V– 5.VI	30.VI –15.VII	86.0(–1,6)	0.17	–	2	Top leaf of flag type
KG–56/17	7.V–31.V	29.VI –11.VII	88.3(+0.7)	0.00	100.00	1	Wide erectile leafs with the most ashen layers
KG–56/20	11.V– 6.VI	1.VII–18.VII	84.3(–3.3)	0.33	–	5	Top leaf of flag type
KG–56/25	9.V– 5.VI	30.VI –13.VII	84.8(–2,8)	0.40	100.00	6	Top leaf of flag type
KG–56/27	11.V– 6.VI	1.VII–13.VII	83.5(–4.1)	0.00	–	4	Medium ashen layer
KG–56/29	10.V– 4.VI	1.VII–12.VII	80.5(–7.1)	0,00	–	6	Medium ashen layer
KG–56/32	11.V– 3.VI	1.VII–11.VII	85.5(–2.1)	0.02	100.00	5	Top leaf of flag type, more ashen layers
KG–56/36	8.V– 2.VI	29.VI –11.VII	88.1(+0.5)	3.30	–	6	Top leaf of flag type, more ashen layers
KG–56/39	10.V– 2.VI	30.VI –11.VII	86.0(–1.6)	0.12	100,00	6	Almost erectile top leaf, few ashen layer
KG–56/Stan.	8.V–31.V	30.VI –14.VII	87.6(St.)	0.32	97.81	4	Top leaf of flag type, few ashen layers

– Type of spike: All without awns. LSD 0.05 , 3.155
– Color of spike: white for all. 0.01 4.232

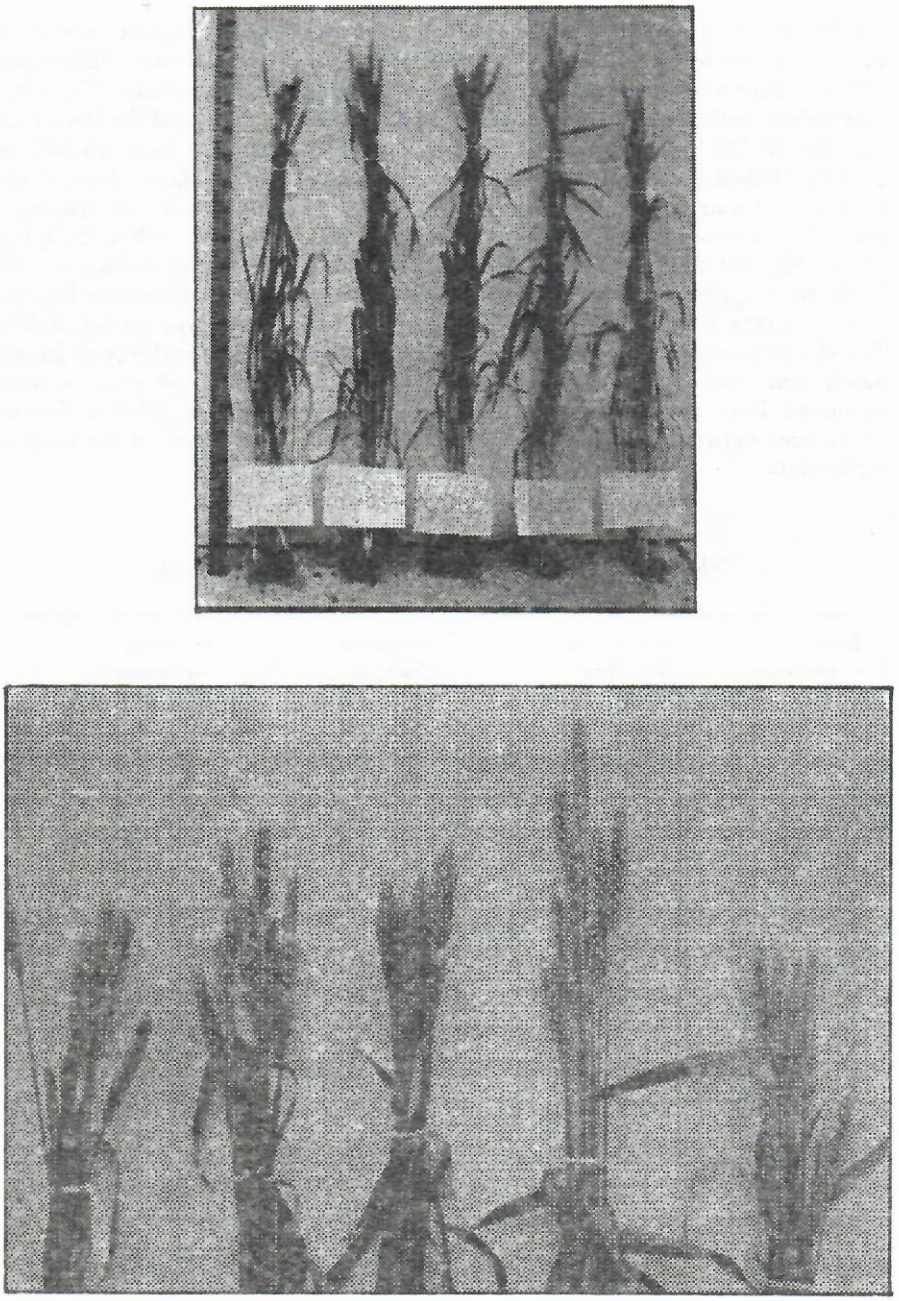

Fig. 1.a) and b) Morphological appearance of the examined sublines in milk ripeness stage. From left to right: Kg. 56/39, Kg. 56/32, Kg. 56/25, Kg. 56/17 and Kg. 56/4.

Kg. 56/36. Lines showed differences in hectolitre mass, too. The highest hectolitre mass was shown by Kg. 56/9 and the lowest by Kg. 56/27. Significant differences between lines were found in the value of sedimentation as a parameter of quality. The highest sedimentation value was measured for line Kg. 56/9 and the lowest for line Kg. 56/36. The content of proteins was found to vary from 14.43% to 17.65%. Gliadin electrophoregram analyses (Fig. 2) of sublines showed the existence of four different genotypes. Line Kg. 56/4 had a specific electropherogram. The second type of electrophoregram was found for sublines Kg. 56/9, Kg. 56/13, Kg. 56/20, Kg. 56/25, Kg. 56/27, Kg. 56/29, Kg. 56/32 and Kg. 56/39. Their electropherograms were similar to the electropherogram of cultivar Kg. 56. The third type was found for line Kg. 56/17, and the fourth type for Kg. 56/36. The electropherograms were evaluated according to the relative mobility of gliadin bands and their color intensities. Electrophoretic formulae of gliadins from examined lines were shown in Table 5. The composition of gliadins showed differences between the lines, which confirmed differences noted on the basis of earlier data.

Table 2. During the vegetation and grain filling periods

Line– – cultivar	Emergence –heading (No. of days) Mean (+St.) 1985/86–88/89	Emergence –ripeness (No. of days) Mean (+ St.) 1985/86–88/89	Heading –ripeness (No. of days) Mean (+ St.) 1985/86–'88/89
KG–54/4	164.0(–0.5)	211.0(+0.5)	47.0(+1.0)
KG–56/9	165.3(+0.8)	211.5(+1.0)	46.3(+0.3)
KG–56/13	165.5(+1.0)	211.8(+1.3)	46.6(+0.3)
KG–56/17	163.0(–1.5)	209.8(–0.7)	46.8(+0.8)
KG–56/20	166.3(+1.8)	213.8(+3.3)	47.5(+1.5)
KG–56/25	165.3(+0.8)	210.3(–0.2)	45.0(–0.1)
KG–56/27	166.3(+1.8)	211.0(+1.5)	44.8(–1.2)
KG–56/29	165.3(+0.8)	210.5(+0.0)	45.3(–0.7)
KG–56/32	165.3(+0.8)	210.8(+.3)	45.5(–0.5)
KG–56/36	163.5(–1.0)	209.0(–1.5)	45.5(–0.5)
KG–56/39	164.5(+0.0)	209.8(–0.7)	45.3(–0.7)
KG–56–St.	164.5(St.)	210.5(St.)	46.0(St.)
LSD .05	5.312	4.838	1.495
LSD .01	7.124	6.488	2.006

For more complete explanation the eight sublines of the second group should be subjected to other analyses (HMW – glutemins, aminoacid analyses, etc.).

Table 3. Registance to some important diseases in field and greenhouse conditions

Line– – cultivar	Puccinia graminis tritici										Fusarium sp. (%) in the field without artificial infection '86/87– –'88/89.	Septoria sp. in the field without artificial infection '85/86– –'88/89 (0–9)
	Type of infection of young plants in greenhouse*				Grown–up plants in the field							
	'87/88 R–11 RRT	'88/89 R–34 RKF	'87/88 R–34 RHT	'88/89 R–214 MJC	'87/88 Cobb	R–11 +R–34 Type of infection	RRT+ RHT Coeficient of infection	'88/89 Cobb	R–34 +R–214 Type of infection	RKF + MJC Coeficient of infection		
KG–56/4	0	0	0	0	40	2	16	40	1	8	1,5	1,0
KG–56/9	3^{+}	–	4^{-}	–	70	4	70	–	–	–	0,0	1,0
KG–56/13	–	–	–	–	–	–	–	–	–	–	–	0,5
KG–56/17	2^{++}	0	4^{-}	0	5	4	5	40	1	2	2,5	0,5
KG–56/20	–	–	–	–	–	–	–	–	–	–	–	1,0
KG–56/25	4^{-}	4^{-}	4	0	80	4	80	50	4	50	1,5	2,0
KG–56/27	–	–	–	–	–	–	–	–	–	–	–	2,0
KG–56/29	–	–	–	–	–	–	–	–	–	–	–	2,0
KG–56/32	4	4	4	0	80	4	80	60	4	60	1,0	1,5
KG–56/36	–	–	–	–	–	–	–	–	–	–	–	1,0
KG–56/39	4^{-}	4	4	2^{+}	80	4	80	60	4	60	0,5	1,5
KG–56/St.	4	–	4	–	70	4	70	–	–	–	3,5	3,5

*Types of infection were determined according to S t a k m a n *et al.* (1962).
Signs + and – were used to designate variations within types.

Table 4. Some parameters of grain quality

Line– – cultivar	1000 kernel weight (g.) Mean (+St.) 1984/85.– 1988/89.	Hectolitre (kg) Mean (+ St.) 1984/85–1988–89.	Sedimentation value (ml.) zeleny Mean (+St.) 1984/85–1988/89.	Crude proteins (%) (+St.) 1986/87. Seed
KG–56/4	45.64(+0.34)	82.54(+0.67)	47.2(–15.6)	15.88(–0.20)
KG–56/9	46.46(+1.16)	83.46(+1.59)	71.2(+ 8.4)	14.43(–1.65)
KG–56/13	45.18(–0.12)	82.92(+1.05)	64.6(+ 1.8)	– –
KG–56/17	48.56(+3.26)	82.26(+0.39)	63.4(+ 0.6)	17.65(+1.57)
KG–56/20	46.82(+1.52)	82.32(+0.45)	64.2(+ 1.4)	– –
KG–56/25	45.78(+0.48	82.56(+0.69)	67.2(+ 4.4)	15.63(–0.45)
KG–56/27	44.32(–0.98)	81.11(–0.76)	65.2(+ 2.4)	– –
KG–56/29	44.24(–1.06)	81.76(–0.11)	67.6(+ 4.8)	– –
KG–56/32	44.64(–0.66)	82.06(+0.19)	64.4(+ 1.6)	15.44(–0.64)
KG–56/36	41.44(–3.86)	82.08(+0.21)	31.4(–31.4)	– –
KG–56/39	44.56(–0.74)	82.02(+0.15)	62.0(– 0.8)	15.37(–0.71)
KG–56/St.	45.30(St.)	81.87(St.)	62.8(St.)	16.08(St.)
LSD .05	2,721	1,132	5,4	
LSD .01	3.628	1,510	7,2	

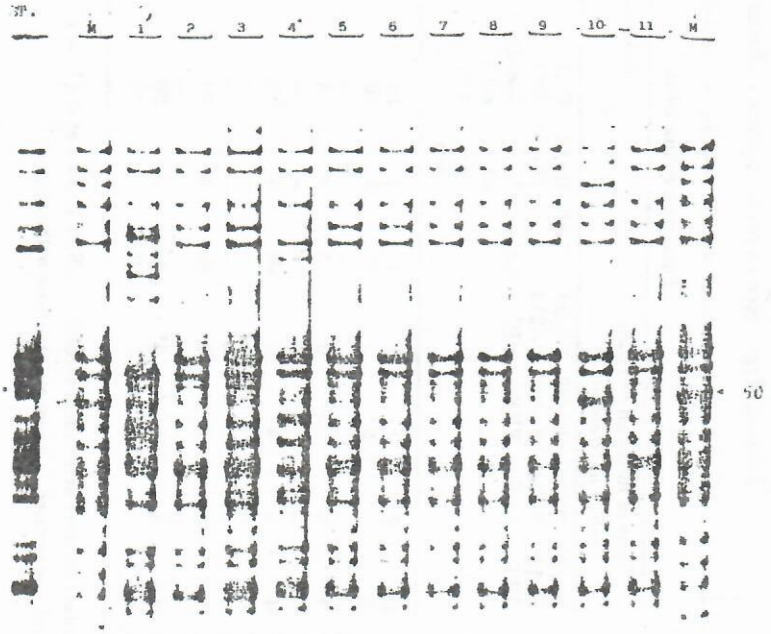

Fig. 2. Electropherograms of gliadins. ST Kragujevačka 56. M–Marquis, 1–Kg. 56/4, 2–Kg. 56/9, 3–Kg. 56/13, 4–Kg. 56/17, 5–Kg. 56/20, 6–Kg. 56/25, 7–Kg. 56/27, 8–Kg. 56/29. 9–Kg. 56/32, 10–Kg. 56/36 and 11–Kg. 56/39

Table 5. Electrophoretic formulas of gliadins for analysed sublines of wheat cultivar KG–56

Subline																																				
KG. 56/4	1	3	3		2	24		22	4	3	1		5	4	4		45		3		52		41			1			3		3		5	3		
KG. 56/9	1	3	2		2	2	3		1	1		5	5	3	2		3		3		41		31			1			3		3		4	3		
KG. 56/13	2	3	3		3	3	5		1	1		5	5	4	3		4		4		52	4	2		2				3		3	5		3	1	
KG. 56/17	1	3	3		3		313		11	1		5	5		22	4		4		2	3		3	2			2	3		3		3		4	31	1
KG. 56/20	1	3	3		2	3	4		1	1		5	5	3	2		3		3		41		31	1				3		3			5	31	1	
KG. 56/25	1	3	2		2	3	4		1	1		5	5	3	2		3		3		41		31	1				3		3			5	31	1	
KG. 56/27	1	3	2		2	3	4		1	1		5	5	3	2		3		3		41		31	1				3		3			4	31	1	
KG. 56/29	1	3	3		2	3	4		1	1		5	5	3	2		3		3		41		3	11				3		3			5	31	1	
KG. 56/32	1	3	2		2	3	4		1	1		5	5	2	2		2		3		31		3	1				2		2			4	31	1	
KG. 56/36	2	2		3	3	2	4	2		1		5	5	1	5		2		3		31		3	2		1		3		3		4		1	1	
KG. 56/39	1	3	3		2	3	5		1	1		5	5	3	2		3		3		42		31	1		1		3		3		5		1	1	
Kragujevačka 56	2	4	3	1	3	3	5		1	2		5	5	3	3		3		3		43		4	2		2		2		3		4		1	1	

Relative band intensity 1 is lightest, 5 is darkest. All gliadin bands were found between 10 and 90 units

CONCLUSION

Cultivar Kg. 56 consists of several genotypes with very similar phenotype. This could be the reason for the good adaptability in different environments.

Considering the results of investiagations of morphological and phisiological traits the existence of 11 genotypes within this cultivar was noted.

Sublines Kg. 56/4 and Kg. 56/17 showed differences for many analysed traits compared with other sublines and cultivar Kg. 56. That is the reason for discarding these lines during the maintenance of the cultivar and for their low frequency. These sublines could be useful for breeding programs because of their desirable traits (resistance to diseases, high technological quality, model of plant, etc.).

The subline Kg. 56/36 is the most different from the other having the worst quality of grain and a short period of vegetation. This subline is the third undesirable genotype within cultivar Kg. 56. Differences between these three sublines and the others were found also by electrophoretic analysis of grain endosperm gliadins.

The other sublines: Kg. 56/9, Kg. 56/13, Kg. 56/20, Kg. 56/25, Kg. 56/27, Kg. 56/29, Kg. 56/32 and Kg. 56/39 showed insignificant and significant differences for some characteristics. These sublines showed very good quality parameters. Compared with cultivar Kg. 56 these lines showed better tehnological quality. Their similarities with each other and with Kg. 56 were confirmed by the similar gliadin composition in the grain endosperm.

Four groups of different genotypes were found by the method of gliadin electropherogram analyses of sublines.

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ISPITIVANJE SUBLINIJA SORTE PŠENICE KRAGUJEVAČKA 56

(Tr. aestivum ssp. vulgare)

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Izvod

U cilju da se ispita polimorfizam unutar sorte Kg. 56 i da se identifikuju superiorni genotipovi za pojedina svojstva proučavana su odabrane sublinije ove sorte. Ispitivanjima su obuhvaćene neke morfološke, fiziološke i tehnološke osobine, kao i otpornost na bolesti (uglavnom osobine visokih heritabilnosti) korišćenjem standardnih metoda. Na osnovu ovih istraživanja ustanovljen je polimorfizam sublinija unutar sorte Kg. 56, a što je potvrđeno i na biohemijskom nivou, analizom glijadina pomoću poliakrilamidne gel elektroforeze.

Metodom 'acid PAGE' Lookhart (1982) identifikovana su 4 različita genotipa u odnosu na sastav glijadina, a na osnovu ostalih analiza identifikovano je 11 sublinija.

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