Screening and Genetical Study for Bread Wheat Gluten Strength Using Solvent Retention Capacity (SRC) Test

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ABSTRACT

This study was carried-out at the Experimental Farm and wheat Laboratory of Sakha Agricultural Research Station, Agricultural Research Center, Kafrelsheikh, Egypt, during 2011/2012 and 2012/2013 seasons. The objectives were to validate the potentiality of SRC test to measure gluten strength differences among the Egyptian bread wheat cultivars, to use SRC technique to screen Sakha bread wheat crossing block for gluten strength and to study the genetics of gluten strength. The two Egyptian bread wheat cultivars Sakha93 and Sids 12 along with their F_1 and F_2 population were used. In addition, 69 bread wheat genotypes from Sakha wheat crossing block were included. The statistical analysis procedure was used according to the regular analysis of variance of completely randomized design. Differences among means were measured using least significant differences. Means, phenotypic variance, environmental variance and broad sense heritability were estimated. The recent results showed the potentiality of SRC test to measure the differences and to screen the Egyptian bread wheat cultivars and /or lines for gluten strength. Depending on SRC results, the two cultivars Sids 12 and Sids 13 and line #25 had strong gluten and it could be used in planed crosses to improve gluten strength. The cultivar Sakha 93 and lines # 55and 86 had weak gluten. SRC test became a routine work at Sakha wheat research program to check gluten strength among high yielding and rust resistance lines selected from the breeding program. The results of genetic study showed partial dominance toward weak gluten. Both additive and non-additive gene effects play an important role in inheritance of this trait. The genetic variance was the main portion of total variance for gluten strength with high estimates of broad since heritability.

Key words: Wheat gluten, Genetic parameters, SRC test.

INTRODUCTION

Bread Wheat (*Triticum aestivum,* L.), as a food crop, consider the most important cereal in Egypt, as well as, many parts of the world. The Egyptian bread wheat research program essentially concentrate on producing high yielding cultivars of biotic and a biotic stresses tolerance. On the other hand, testing for wheat quality is conducted only during the final steps before releasing the new cultivar. Availability of simple and friendly method for evaluation and selection of high quality bread wheat genotypes in early generation of the breading program might increase the efficiency of quality improvement. Wheat flour quality is an important factor affecting the preference of Egyptian farmers. Wheat flour quality can be considered as a combination of performance and conformance (also known as consistency), which is impacted by the four major factors of genetics, agronomics, milling, and baking (Kweon *et al.,* 2011). Evaluating flour quality is an essential task for breeders, millers, and bakers in selecting good quality wheat cultivars with optimized performance.

Originally, SRC testing was created and developed for evaluating soft wheat flour functionality. The major flour functional components are damaged starch, gluten proteins, and arabinoxylans (also known as pentosans). The SRC test is a solvation assay for flours that is based on the enhanced swelling behavior of individual polymer networks in selected single diagnostic solvents to predict the functional contribution of each individual flour component. The SRC method is increasingly used by wheat breeders, millers, and bakers, as well as by cereal and other research scientists (Pareyt *et al.,* 2008; Linlaud *et al.,* 2009; Kongraksawech *et al.,* 2010; Pareyt 2010; Duyvejonck *et al.,* 2011a; Duyvejonck *et al.,* 2011b; Jazaeri *et al.,* 2011; Moses and Dogan 2011).

The objectives of this study are to I) Validate the ability of SRC test to measure gluten strength differences among Egyptian bread wheat cultivars, II) Use SRC technique to screen Sakha bread wheat crossing block and III) Study the genetics of gluten strength.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm and the Laboratory of wheat department of Sakha Agricultural Research Station, Agricultural Research Center (ARC), Kafrelsheikh, Egypt, during 2011/2012 and 2012/2013 seasons.

Plant material

Screening test included 69 bread wheat genotypes from Sakha bread wheat crossing block (Table 1), besides the two bread wheat cultivars Sids 12 and Sakha 93 were screened for gluten strength using SRC test. For genetic study, the two parental cultivars Sids 12, Sakha 93, F_1 and F_2 population were used. The kernels of five, five, five and 75 individual plants from P_1, P_2, F_1 and F_2, respectively, were randomly harvested, air dried, milled and used for SRC test.

Laboratory experiments

Three SRC experiments were done at Sakha research station's laboratory, wheat department. The first experiment was to differentiate between the two Egyptian bread wheat cultivars Sakha 93 and Sids 12. The second experiment was to screen 69 bread wheat genotypes from Sakha bread wheat genotypes used for crosses (Sakha crossing block). The third experiment was to study genetics of bread wheat gluten strength using SRC. Samples were milled using cyclone sample mill. Four solvents were used for the SRC test: 1) freshly prepared 5% w/w lactic acid (LA), 2) 5% w/w sodium carbonate (Na_2Co_3), 3) 50% w/w sucrose (Suc), prepared the day before SRC testing, 4) deionized or distilled water. The SRC test is a relatively simple and user-friendly method (AACC International Approved Method 56-11.02, 2010) with minor modification in flour sample size from 5g to 1g (Bettge *et al*., 2002).

Table 1: Genotype, cross name, selection history and origin of representative genotypes from Sakha wheat crossing block season 2011/2012.

Genotype	Cross name and selection history	Origen
GIZA 171	SAKHA 93 / GEMMEIZA 9 S.6-1GZ-4GZ-1GZ-2GZ-0S	EGYPT
SAKHA 93	SAKHA92/TR810328 S.8871-1S-2S-1S-0S	EGYPT
SAKHA 94	OPATA/RAYON//KAUZ CMBW90Y3180-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S.	CIMMYT
GEMMIZA 11	BOW"S"/KVZ"S"//7C/SER182/3 /GIZA168/SAKHA 61 GM7892-2GM-1GM-2GM-1GM-0GM	EGYPT
CHANDWEEL 1	SITE/MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC CMSS93B00567S-72Y-010M-010Y-010M-3Y-0M-0HTY-0SH	CIMMYT
SIDS 12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S" /6/MAYA /VUL // CMH74A.630 / 4*SX SD7096-4SD-1SD-1SD-0SD	EGYPT
SIDS 13	KAUZ"S" //TSI / SNB"S" ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP-0SD	ICARDA
MISR 1	OASIS / SKAUZ // 4*BCN /3/ 2*PASTOR CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S	CIMMYT
MISR 2	SKAUZ / BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0S	CIMMYT
Line # 11	BL1133 /3/CMH 79A.955*2/ CNO 79//CMH 79A.955 /BOW"s"/4/GIZA 164/ SAKHA61 S.13737-3S-1S-1S-0S	EGYPT
Line # 16	KAUZ / ATTILA /7/ KVZ /4/ CC / INIA /3/ CNO // ELGAU / SON 64 /5/ SPARROW "S" / BROCHIS "S" /6/ BAYA "S" / IMU S. 15563-9S-3S-1S-0S	EGYPT
Line # 20	SAKHA 12 /5/ KVZ // CNO 67 / PJ 62 /3/ YD "S" / BLO "S" /4/ K 134 (60) / VEE /6/ HUBARA-1 S. 15868-3S-1S-2S	EGYPT
Line # 23	SAKHA 8 / YECORA ROJO	
Line # 25	SAKHA 94 // KAUZ / PASTOR S. 15962-2S -0SY-1S	EGYPT
Line # 45	DVERD 2 / AE - SQUARROSA (214)// 2* BCN CMSS92Y0186-6M-2Y-3M-010Y-1KBY-8M-0Y	CIMMYT
Line # 55	SAKHA 93 / GIZA 168	EGYPT
Line # 58	TOBA97/ATTILA CMSS97M05753S-020Y-030M-020Y-040M-48Y-3M-0Y	CIMMYT
Line # 72	KAUZ/PASTOR//BAV92/RAYON CMSS00M02400S-030M-030WGY-030M-13M-0Y-0NUB	CIMMYT
Line # 80	WBLL1*2/BRAMBLING CGSS01B00062T-099Y-099M-099M-099Y-099M-73Y-0B-0S	CIMMYT
Line # 86	CMH83.2517 / ELVIRA /6/ CMH79A.955 /4/ AGA /3/ 4*SN64 / CNO67 // INIA66 /5/ NAC	CIMMYT

SRC Procedure:

1. Flour, whole meal, 1.0 g is weighed into a 10 mL conical-bottom centrifuge tube.
2. 5.0 g of a selected solvent is added to the flour.
3. This flour solvent mixture is tapped on the bench and then mixed well with hand shaking (every 5min) for 20 min to disperse the flour without lumps.
4. The flour suspension is centrifuged at 1,000×*g* for 15 min using IEC- HN-SII centrifuge.
5. The supernatant is discarded and the tube is allowed to drain for 10 min.
6. The weight of the swollen pellet is then measured.

The SRC value is calculated based on 14% flour moisture content and expressed as gram solvent per gram flour. The gluten performance index (GPI) was calculated using the following formula; GPI = LA value / (Na_2Co_3 value + Suc. value). The four SRC values were multiplied by ten to facilitate data analysis. Kweon et al., 2011 reported SRC estimates of ≤ 5.1, ≥8.7, ≤ 6.4 and ≤ 8.9 for water, LA, Na_2Co_3 and Suc, respectively, are slandered for cookies and crackers. Meanwhile, sponge and dough products standard SRC estimates are ≤ 5.7, ≥10.0, ≤ 7.2 and ≤ 6.9 for water, LA, Na_2Co_3 and Suc, respectively.

Statistical analysis

The statistical analysis procedure was used according to the regular analysis of variance of completely randomized design (CRD) and the differences between means were measured using least significant differences (LSD) test, in this respect GenStat 14th edition was used. Means, phenotypic variance (VF_2), environmental variance (VF_1+VP_1+VP_2/3) and broad sense heritability were measured according to Allard 1960.

RESULTS AND DISCUSSION

SRC provides a measure of solvent compatibility for the three functional polymeric components of flour (gluten, damaged starch, and pentosans); 5% w/w lactic acid in water with gluten, 5% w/w sodium carbonate in water with damaged starch and 50% w/w Sucrose in water with pentosans.

Analysis of variance for SRC estimates showed highly significant differences between the two Egyptian bread wheat cultivars in LA, Na_2Co_3, Suc and water estimates (Table 2).

Sakha 93 SRC LA estimate (9.512) was lower than that of Sids 12 (11.224). The same trend was observed for the other three estimates; $NaCo_3$, Suc and GPI in both cultivars (Table 3). These differences between the two cultivars indicated that the SRC test had good ability to measure the differences between the two bread wheat genotypes in their gluten strength.

Table 2: Analysis of variance for Solvent retention capacity (SRC) estimates for the two Egyptian bread wheat cultivars Sids 12 and Sakha 93 season 2011/2012.

SRC estimate	Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	Calculated F	F probability	cv%
LA	Genotyps	1	7.3308	7.3308	63.67	< 0.001	3.3
	Residual	8	0.9212	0.1151			
	Total	9	8.2519				
Na_2Co_3	Genotyps	1	8.3832	8.3832	11.11	0.01	7.2
	Residual	8	6.0351	0.7544			
	Total	9	14.4184				
Suc	Genotyps	1	4.1474	4.1474	6.11	0.039	6.3
	Residual	8	5.4274	0.6784			
	Total	9	9.5747				
Water	Genotyps	1	4.9224	4.9224	17.62	0.003	5.7
	Residual	8	2.2343	0.2793			
	Total	9	7.1567				

LA= 5%w/w lactic acid, Na_2Co3 = 5% w/w Sodium carbonate, Suc = 50% w/w sucrose

Table 3: SRC values for the two Egyptian bread wheat cultivars Sids 12 and Sakha 93 season 2011/2012.

Genotype	SRC[a] values (g solvent/g flour)				GPI
	LA	Na_2Co_3	Suc	water	
Sids 12	11.224	12.95	13.779	9.947	0.46
Sakha 93	9.512	11.119	12.491	8.544	0.40
LSD 0.05	0.495	1.267	1.201	0.771	

[a]SRC values were multiplied by ten to facilitate data analysis, LA= 5%w/w lactic acid, Na_2Co_3 = 5% w/w Sodium carbonate, Suc = 50% w/w sucrose and GPI (gluten performance index = LA / (Na_2Co_3 + Suc)).

Ram *et al.* (2005) reported the application of SRC test for predicting mixing properties of wheat flours from 192 genotypes. They concluded that, whole-meal SRC values, together with grain protein content, could be used to screen early-generation for farinograph water absorption and there is a high correlation between SRC values and functional properties.

Flour for bread production generally requires high water absorption, good gluten strength and relatively high damaged starch and pentosans. Flour for cookie production generally requires low water absorption, minimal gluten strength and low damaged starch and pentosans. (Kweon *et al.,* 2011). So, the obtained results in this investigation showed that, Sids 12 cultivar is suitable for bread production while Sakha 93 cultivar is suitable for cookies industry.

The SRC test was used to characterize gluten strength of 69 genotypes of Sakha bread wheat crossing block season 2011/2012. Analysis of variance for SRC estimates showed highly significant differences among the 69 bread wheat genotype from Sakha crossing block season 2011/ 2012 (Table 4).

The results showed that the bread wheat cultivar Sids 13 and line #25 had the highest estimates of LA and GPI. The estimates were higher than that obtained for the cultivar Sids 12 high gluten strength (Table 3 and 5). On the other side, Lines # 55and 86 had the lowest estimates of LA and GPI. These results indicate that the cultivar Sids 13 and Line #25 had strong gluten and can used to improve gluten strength in the Egyptian bread wheat breeding program.

Table 4: Analysis of variance for solvent retention capacity (SRC) estimates of 69 bread wheat genotype from Sakha crossing block season 2011/ 2012.

SRC estimates	Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	Calculated F	F probability	cv%
LA	Genotypes	68	188.952	2.779	33.880	< 0.001	2.9
	Residual	69	5.658	0.082			
	Total	137	194.610				
Na_2Co_3	Genotypes	68	303.172	4.458	3.650	< 0.001	10.5
	Residual	69	84.206	1.220			
	Total	137	387.377				
Suc	Genotypes	68	154.886	2.278	2.140	< 0.001	9
	Residual	69	73.475	1.065			
	Total	137	228.361				
Water	Genotypes	68	100.397	1.476	3.770	< 0.001	7.4
	Residual	69	26.993	0.391			
	Total	137	127.390				

LA= 5%w/w lactic acid, Na_2Co_3 = 5% w/w Sodium carbonate, Suc = 50% w/w sucrose

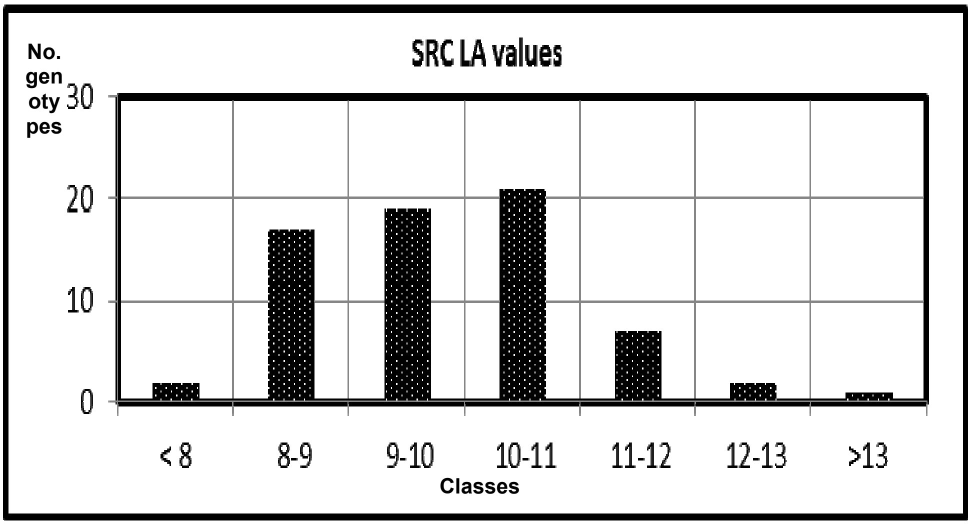

Fig. 1: Frequency distribution of SRC lactic acid values for 69 genotypes of Sakha crossing block season 2011/ 2012.

Table 5: SRC values for representative genotypes of the 69 bread wheat genotype from Sakha crossing block 2011/ 2012.

Genotype	SRC[a] values (g solvent/g flour)				GPI
	LA	Na_2Co_3	Suc	water	
Sids 13	13.408	11.892	12.28	9.172	0.55
Line #25	12.924	12.66	12.076	10.42	0.52
Shandweel 1	11.36	12.488	13.208	9.876	0.44
Line #72	10.652	13.84	12.324	9.676	0.41
Misr 1	10.468	12.396	12.656	9.532	0.42
Line #58	10.42	13.472	13.444	10.452	0.39
Sakha 94	10.312	12.14	12.288	9.488	0.42
Line #20	9.876	10.588	8.82	8.324	0.51
Misr 2	9.572	10.896	11.116	8.904	0.43
Line #16	9.408	9.968	13.804	8.128	0.4
Line #80	9.148	14.944	10.92	8.512	0.35
Line #11	8.992	10.252	8.456	8.064	0.48
Line #23	8.68	8.36	11.308	8.184	0.44
Gemmeiza 11	8.56	9.272	12.3	7.888	0.40
Line #45	8.428	8.556	11.576	7.432	0.42
Giza 171	8.176	8.9	10.048	7.716	0.43
Line #55	7.748	8.848	10.944	7.268	0.39
Line #86	7.716	9.144	10.812	7.58	0.39
$LSD_{0.05}$	0.571	2.204	2.059	1.248	

[a]SRC values were multiplied by ten to facilitate data analysis, LA= 5%w/w lactic acid, Na_2Co_3 = 5% w/w Sodium carbonate, Suc = 50% w/w sucrose and GPI (gluten performance index = LA / (Na_2Co_3 + Suc)).

SRC mean values, standard error, coefficient of variation and least significant differences for the two bread wheat cultivars Sakha 93 and Sids 12 and their F_1 and F_2 were illustrated in Table 6. The results indicated that SRC values for the cultivar Sids 12 differed significantly from that of Sakha 93 for LA, Na_2Co_3 and water.

F_1 LA mean value (10.063) was between the mid parent value (10.368) and the weak gluten parent value, Sakha 93 (9.512), Table 6 and Fig. 2 (a) indicating partial dominance toward the weak gluten. Regarding the distribution of F_2, Fig.2b, most of F_2 individuals (60%) were less than mid parent support the partial dominance toward weak gluten. Transgressive segregation was observed, where 5% of the studied F_2 population recorded LA values less than that of the weak gluten parent (Sakha 93), while 13% were higher than the strong gluten parent (Sids 12). These results indicate that both additive and non-additive gene effects are involved in the inheritance of this trait.

SRC values maximum and minimum, environmental and genotypic variances and broad sense heritability for Sakha93 and Sids 12 and their F_1 and F_2 population are illustrated in Table 6. For LA the genotypic variance was the main portion of phenotypic variance, where, it gave high estimate of broad sense heritability (0.90). On the other hand, the magnitude of environmental variance was higher than genotypic variance for Na_2Co_3 and Suc estimates. These results were reflected in medium estimates for the broad sense heritability (0.45 and 0.40), (Table 7). Anna and Cantrell 1986 estimated broad sense heritability in two durum wheat crosses and reported that the heritability of gluten strength was moderately to high.

Table 6: SRC mean values, standard error (± se) and least significant differences (LSD) of the two wheat cultivars Sakha 93, Sids 12 and their F_1 and F_2 populations.

Population	Parameter	SRC[a] values (g solvent/g flour)			
		LA	Na_2Co_3	Suc	Water
Sakha 93	Mean	9.512	11.119	12.491	8.544
	± Se	0.173	0.346	0.367	0.194
Sids 12	Mean	11.224	12.95	13.779	9.947
	± Se	0.126	0.426	0.37	0.272
Mid parent mean		10.368	12.0345	13.135	9.2455
F_1	Mean	10.063	10.549	13.363	8.878
	± se	0.159	0.252	0.408	0.307
F_2	Mean	10.441	11.344	13.519	9.717
	± Se	0.126	0.121	0.128	0.094
$LSD_{0.05}$		0.528	1.114	1.397	1.001

[a]SRC values were multiplied by ten to facilitate data analysis, LA= 5%w/w lactic acid, Na_2Co_3 = 5% w/w Sodium carbonate and Suc = 50% w/w sucrose .

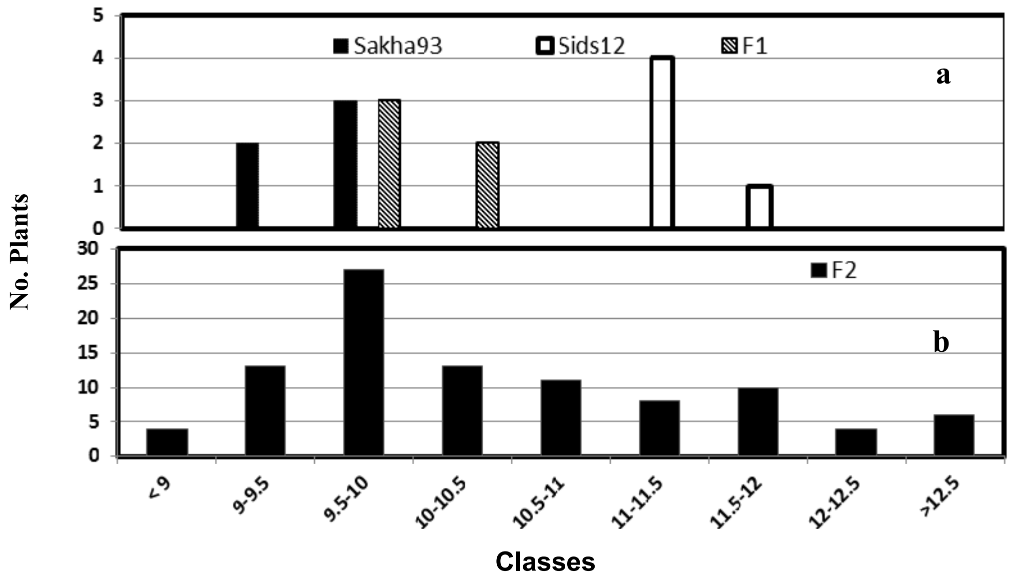

Fig. 2: Distribution of SRC lactic acid (LA) values in parental and F1 (a) and F2 (b) populations of bread wheat cross Sakha 93 x Sids 12.

Table 7: SRC maximum (Max), minimum (Min) and variance (V) values of the two bread wheat cultivars Sakha 93, Sids 12 and their F_1 and F_2 populations.

Population	Parameter	SRC[a] values (g solvent/g flour)			
		LA	Na_2Co_3	Suc	water
Sakha 93	Max	9.990	11.848	13.280	9.024
	Min	9.001	10.268	11.320	7.936
	V	0.150	0.600	0.673	0.188
Sids 12	Max	11.680	14.536	15.008	10.816
	Min	11.001	12.080	13.128	9.344
	V	0.080	0.909	0.683	0.371
F_1	Max	10.476	11.312	14.440	9.856
	Min	9.696	9.960	12.120	8.224
	V	0.126	0.318	0.832	0.471
F_2	Max	12.864	13.76	15.744	11.96
	min	8.336	7.712	10.856	8.104
	V	1.182	1.107	1.223	0.664
Environmental variance		0.119	0.609	0.730	0.343
Genotypic variance		1.063	0.498	0.493	0.320
Broad sense heritability		0.90	0.45	0.40	0.48

[a]SRC values were multiplied by ten to facilitate data analysis, LA= 5%w/w lactic acid, Na_2Co3 = 5% w/w Sodium carbonate and Suc = 50% w/w sucrose.

Guttieri *et al.,* (2001) used SRC test for 26 flour samples of soft white spring wheat genotypes grown in seven irrigated environments in Idaho. They reported that, genotype differences were more important than crop irrigation and fertility management treatments, and genotype × environment interactions were negligible. Guttieri *et al.,* (2002) and Guttieri and Souza (2003) reported that, milling and baking quality could be improved through manipulation of flour components using SRC selection in wheat breeding program.

COCLUSSION

The results in this investigation showed the ability of SRC test to measure the differences and to screen the Egyptian bread wheat cultivars and /or lines for gluten strength. Depending on SRC data, the two bread wheat cultivars Sids 12 and Sids 13 and line #25 could be used in planed crosses to improve gluten strength. The cultivar Sakha 93and lines # 55and 86 had weak gluten. The results of genetic study showed partial dominance toward weak gluten. Both additive and non-additive gene effects play an important role in inheritance of gluten strength. The high estimates of broad sense heritability indicate the effectiveness of improving gluten strength throw early generation selection.

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الملخص العربى

دراسة وراثة قوة الجلوتين في قمح الخبز باستخدام اختبار القدرة على الاحتفاظ بالمذيب(SRC)

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[١]قسم بحوث القمح – معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعية– مصر

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اجري هذا البحث في المزرعة البحثية ومعمل قسم بحوث القمح بمحطة البحوث الزراعية بسخا– مركز البحوث الزراعية– مصر في الموسمين ٢٠١١/٢٠١٢و ٢٠١٢/٢٠١٣ . تهدف الدراسة الي ١) التحقق من قدرة اختبار SRC على قياس الاختلافات في قوة الجلوتين بين اصناف قمح الخبز المصرية. ٢) فرز ٦٩ سلالة وصنف قمح خبز من الاباء المستخدمة في التهجين موسم ٢٠١١/ ٢٠١٢ من حيث قوة الجلوتين. ٣) دراسة وراثة قوة الجلوتين في دقيق قمح الخبز. واثبتت نتائج هذه الدراسة قدرة اختبارSRC على قياس الاختلافات بين اصناف وسلالات قمح الخبز المصرية من حيث قوة الجلوتين. كما اظهرت النتائج ان الصنفين سدس ١٢ وسدس ١٣ والسلالة رقم ٢٥ ذات جلوتين قوي، ويمكن استخدامها في تحسين صفة قوة الجلوتين اقماح الخبز المصرية. دلت نتائج الدراسة ان الصنف سخا ٩٣ والسلالتين رقم ٥٥ و٨٦ ذات جلوتين ضعيف. يستخدم اختبار(SRC) بشكل روتيني في اختبار قوة الجلوتين للسلالات الناتجة من برنامج تربية قمح الخبز بسخا والمرشحة لتجارب تقييم المحصول على المستوى القومي. اظهرت نتائج دراسة وراثة صفة قوة الجلوتين وجود سيادة جزئية تجاه الجلوتين الضعيف. كما ظهرت اهمية كل من الفعل الجيني المضيف وغير المضيف في وراثة هذه الصفة. وكان التباين الوراثي يمثل القسم الاكبر من التباين الكلي وانعكس ذلك علي القيم العالية لدرجة التوريث بمعناها الواسع.