ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol.45(2): 749-757, (2007).

GRAIN SURFACE SCAN AND CYTOLOGICAL STUDIES IN A SELECTED WHEAT LINE POSSESSING THE 4BS.4BL-5RL TRANSLOCATION.

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ABSTRACT

The study was carried out for the aim of combining the best quantitative and qualitative characters of the Egyptian hexaploid wheat (Sides cultivar) with the increasing the efficiency of copper absorption. The observed means of the studied characters for the selected line are nearly within the range of the Sides 6 parent. Meanwhile, it was found that the grain weight per spike is lower (4.13 gm) than that of the Sides 6 (5.20 gm). Similar results in case of 1000-kernel weight, as it reached 48.50 gm in case of the selected line while it reached 59.45 gm in the Sides 6.

C-banding analysis showed that the line was homozygous for a wheat-rye translocation involving the short and long arms of the wheat chromosome 4B and the segment of the long arm of rye chromosome (5RL). The long arm of the wheat-rye translocated chromosome revealed the distinctive C-banding patterns of rye 5RL, which have strong bands in the terminal and subterminal regions.

Stereo microscope and Scanning electron microscopy of dry grains revealed morphological details of grain surface structure. The grains of Pc 62 cultivar showed narrower size than the grains of Sides 6 or the selected line. The hairy end of the Sides 6 grains is broader than the hairy end of the Pc 62. The hairy end of the selected line grains is nearly closed to Sides 6 grains.

INTRODUCTION

Several lines showing the copper efficiency character of rye and carrying different wheat-rye translocations including 5RL of rye have been isolated. Two different 4BS.5RL translocations arose spontaneously as cornell wheat selection (82a1-2-4-7) carrying a segment of 5RL from Rosen rye joined to 4B chromosome (Driscoll and Sears,1965), and Viking wheat with a hairy peduncle and carrying a segment of 5R (Riley *et al.*,1970). Another type of translocation with a segment of 5RL from Imperial rye translocated into wheat chromosome arm (5BS) was isolated after irradiation treatment (Sears,1967). Schlegel *et al.*, (1993) revealed that in the translocation Line VHN, the copper efficiency gene (Ce) was located in a relatively small region of the distal proportion of the donor chromosome 5R. The production of wheat lines with small rye chromosome segments has been achieved. koebner and shepherd (1986a and b) studied a wheat–rye translocation including 1DL-1RS and stated that reduction in yield and quality in these wheat–rye translocation lines was due to either the presence of deleterious rye genes which were transferred at the same time as the target gene or to the loss of desirable wheat genes. They suggested that induction of recombination between rye chromatin and its wheat homoeologue could overcome this type of problem, either through the removal of deleterious genes from the rye segment and/or the re-incorporation of the wheat genes of importance to bread-making quality.

Copper is an important micronutrient for growth and development of plants. Its role in plants appears to be most notably for the production of viable pollen and therefore affects grain yield in cereal crops (Brown and Clark,1977; Graham *et al.*,1987; Grundon 1991 and Leach and Dundas, 2006). Harry and Graham (1981) used green house pot experiments to study the effects of copper deficient soil on rye, wheat and triticale. They revealed that rye gave maximum yield, irrespective of the copper status of the soil, triticale gave an intermediate yield and wheat no yield when severely deficient conditions are applied compared with the control (i.e. maximum yield). Wheat is severely affected by copper deficiency and is completely male sterile under conditions when copper levels are still sufficient for near maximal yield of rye (Graham 1975,1976,1981,1984;Owuoche *et al.*,1994 and Soon *et al.*,1997), but not to the extent to which rye is efficient. A characteristic of copper deficiency is a significant loss in grain yield of up to 20%, without prior symptoms (Graham and Nambiar, 1981).

Chromosome identification has long been important for gene manipulation in plant research and breeding programs. Many methods can be applied to identify chromosomes in cereals. Among these methods, cytological techniques are the most commonly used. Differential staining techniques (chromosome banding) can provide precise identification of all the chromosomes in many Triticeae species (Sybenga, 1983; Lukaszewski and Gustafson 1983; Gill *et al.*, 1991 and Kakeda *et al.*, 1991). Today, many chromosome segments and structural variations can be detected.

The present paper, therefore, concentrates on the cytological proof of the presence of an alien segment of rye chromosome (5R) on wheat chromosome 4B in a new promising line derived from hybridization between Sids 6 and Pc 62. There has been little effort to enable utilization from rye genes in the development of wheat lines with high performance in copper absorption. Moreover, scanning the wheat grains through using the surface scan electron microscopy for the three genotypes.

MATERIALS AND METHODS

This investigation was carried out at the agricultural research station of Moshtohor, Faculty of Agriculture, Benha University. The material used in this study was one local hexaploid wheat cultivar; Sids 6 provided kindly from the Ministry of

Agriculture, Dokky, Egypt (Prof. Dr. Abd El-Salam Gomaa) and wheat-rye translocation cultivar; Pc 62 harboring a terminal segment of the rye chromosome (5RL) on the wheat chromosome (4BS-4BL) provided kindly from Germany (Prof Dr Hassan Sherif).

From previous studies, plants belonging to Sides 6 wheat cultivar (*Triticum aestivum L.*) were crossed with plants of Pc 62 wheat cultivar (*Triticum aestivum L.*) including wheat-rye translocation (4BS-4BL-5RL). Hybrid of the F_1 was pollinated using plants of Sides 6. During 2001/2002 season, the progeny of the BC_1F_1 was selfed for two successive seasons. Plants similar to Sides 6 cultivar with hairy peduncle were selected during each of the two seasons. The progeny of each selected plant was grown separately for evaluation. Data were evaluated in parents and the progeny of the selected plants as the following:

1. Evaluation of some morphological and yield characters; plant height, flowering date, maturity date, number of spikelets per spike, grain yield per main spike and 1000-kernel weight were evaluated for parents and the progeny of the selected line.
2. Cytological studies: C-banding technique was applied according to Gill *et al.*, 1991 that modified after Giraldez *et al.*, 1979.
3. Grain surface scan and macro morphology: The detailed surface scan attribute features were examined by Stereo microscope and Scanning Electron Microscopy (SEM) using suitable magnification (100 µm for surface scan). The dry grains were mounted on copper stubs and coated with a thin layer of gold palladium in a sputter coater unit, Polaron E5000. Scanning was done on JEOL-JSM T(1000) Model Scanning Electron Microscope at the central lab of National Research Center, Dokky, Giza. The magnification power was expressed under each SEM photograph. Each sample is represented by three micrographs: a. complete grain (dorsal and ventral view). b. surface scan of epidermal grain coat (surface view) and c. surface scan of the hairy end. The sputter coating procedure: Edwards England S 150 A (*) sputter coating had been used to coat samples with golden layer before being viewed in the SEM that make surface of samples very reflective.

RESULTS AND DISCUSSION

The study was carried out for the aim of combining the best quantitative and qualitative characters of the Egyptian hexaploid wheat (Sides cultivar) with the increasing the efficiency of copper absorption. This character was detected in the German Pc 62 as it carries gene or genes responsible for increasing the efficiency of copper efficiency (5RL). Schlegel *et al.*, 1993 revealed that these genes were linked to a marker gene control the presence of pubescence on the spike neck (hairy peduncle). Table (1) revealed the observed means and standard errors of the studied characters (morphological and yield characters) for the two parents; Sides 6 and Pc 62 in addition to the progeny of the selected line. All the studied characters are nearly within the range of the Sides 6 parent. For the plant height; it was 115.00 cm for the selected line compared to 112.00 cm in case of Sides 6. Concerning flowering date; it was 89.10 day compared to 84.17 in case of Sides 6. In case of maturity date; it reached 154.00 days as compared to 153.25 days in Sides 6. Concerning spike length, it was 18.50 cm while it reached 17.35 cm in the Sides 6. In case of spikelets number, it was 23.95 while it was 23.45 in the Sides 6. Meanwhile, it was noted that the grain weight per spike is lower (4.13 gm) than that of the Sides 6 (5.20 gm). Similar results in case of 1000-kernel weight, as it reached 48.50 gm in case of the selected line while it reached 59.45 gm in the Sides 6. The reduction in grain yield and 1000-kernel weight is expected and in agreement with the finding of Schlegel *et al.,* 1993 who reported that Viking (-5RL) yielded higher than the translocation line; VHN (+5RL) in copper sufficient conditions in a pot assays, whereas under deficient conditions, the yield of the control was reduced 97 % when compared with the translocation line. At the meantime, Graham *et al.,* 1987 backcrossed the 4BS.4BL-5RL translocation into several locally adapted cultivars and were able to improve yield by more than 100 % on average. The 4BS.4BL-5RL translocation lines were found to be the highest yielding of the lines examined.

Table (1): Means and standard errors of seven morphological and yield characters in two hexaploid wheat parents (Sids 6 and Pc 62) and the selected line.

Geno-types	Flowering date	Maturity date	Plant height	Spike length	Spikelets number	Grain yield/Spike	1000-kernel weight
Sids 6	84.17± 2.20	153.25± 3.70	112.00± 2.93	17.35± 0.10	23.45± 0.64	5.20± 0.06	59.45± 1.10
The selected line	89.10± 3.05	154.00± 4.16	115.00± 3.82	18.50± 0.11	23.95± 0.73	4.13± 0.02	48.50± 0.95
Pc 62	121.95± 2.60	160.00± 4.40	138.50± 3.24	10.65± 0.02	21.15± 0.85	2.05± 0.00	31.85± 0.85

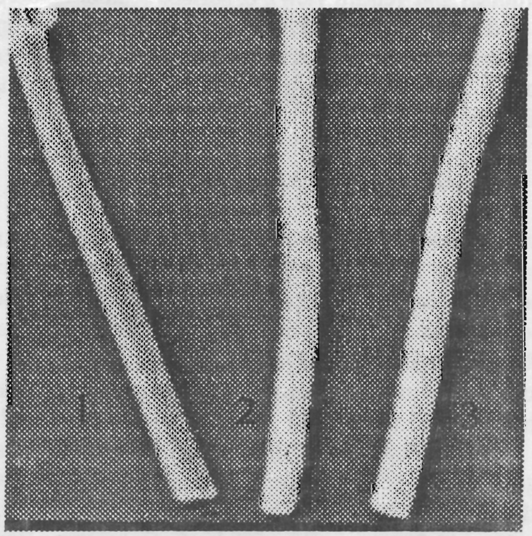

Fig. (1): Show the hairy neck on the stem of Pc 62 cultivar (3) and the selected line (2) compared to Sides 6 without the hairy neck (1).

Chromosomal analysis:

As shown in Fig. 2, C-banding of mitotic metaphase permitted the identification of rye chromatin found in the selected line obtained from crossing between Sides 6 and Pc 62 cultivars. The selected line revealed a chromosome number of 2n = 42. C-banding analysis showed that the line was homozygous for a wheat-rye translocation involving the short and long arms of the wheat chromosome 4B and the segment of the long arm of rye chromosome (5RL). The long arm of the wheat-rye translocated chromosome revealed the distinctive C-banding patterns of rye 5RL, which have strong bands in the terminal and subterminal regions. These banding patterns were identical with those of the 4BS.4BL-5RL chromosome at several wheat cultivars by Schlegel *et al.*, 1991.

The cytological results demonstrate the presence of a 4BS.4BL-5RL translocation in the hairy nick spikes of the selected line similar to that found in the Pc 62 cultivar. Its size, somatic stability and adaptive genetical behavior as well as its copper efficiency can contribute to the development of commercial copper efficient wheat cultivar by introgression of the alien rye segment. Therefore, a backcross program has been initiated to transfer this character into locally-adapted Egyptian wheats.

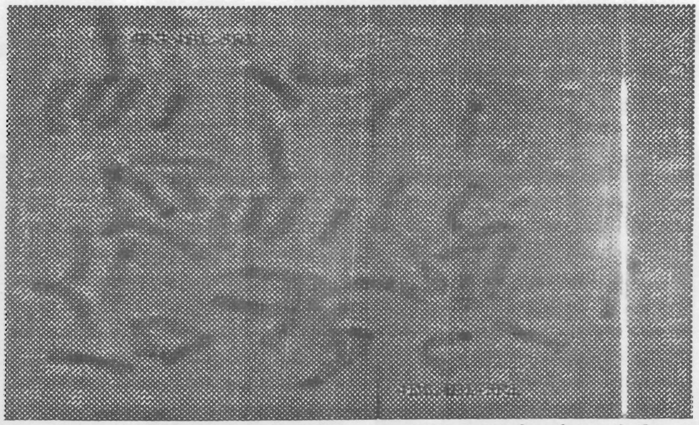

Fig. (2): Cytogenetic analysis of the chromosome constitution of the selected line showing the chromosome translocation (4BS.4BL-5RL).

Stereo microscope of dry grains revealed morphological details of grain surface structure. Figure 3 revealed that plump grains from the two parents and the experimental line had smooth surfaces which could be easily distinguished from buckled and wrinkled surfaces of Triticale. The grains of Pc 62 cultivar showed narrower size than the grains of Sides 6 or the selected line. The hairy end of the Sides 6 grains is broader than the hairy end of the Pc 62. The hairy end of the selected line grains is nearly closed to Sides 6 grains (Figure 4). Scanning electron microscopy revealed that all grain surfaces of the three genotypes have reticulate sculpture (Barthlott, 1984 and Loutfy, 1992 and Salama, 2003). The scan surface of the Pc 62 grains is narrower than the surface of Sides 6 and the selected line. There were, however, no obvious differences in scan surfaces between selected line grains and those of the Sides 6 (Figure 5).

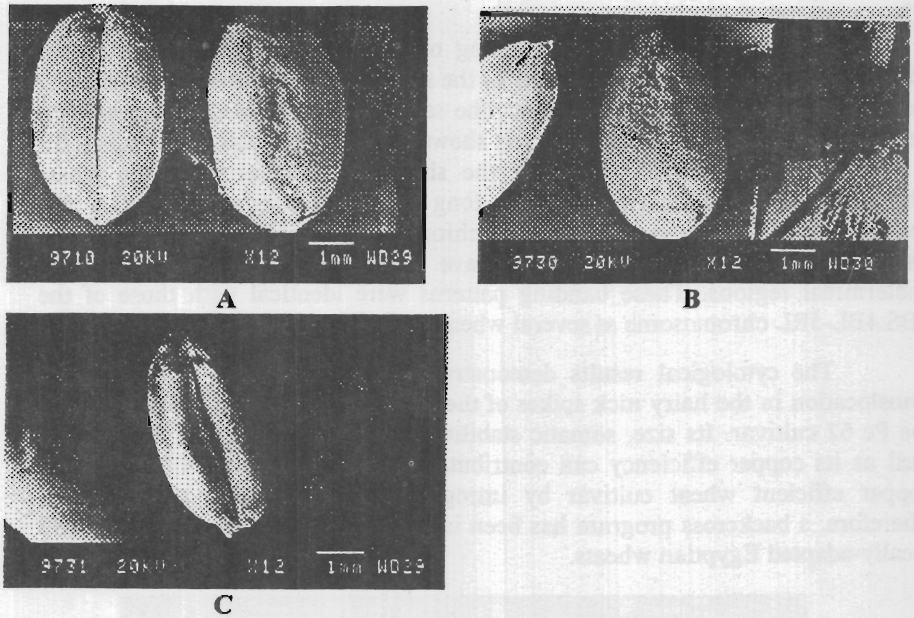

Fig. (3): Stereo microscope micrographs of grains in the three genotypes (smooth surface and prominent embryo). A. Sides CV., B. the selected line and C. Pc 62 CV.

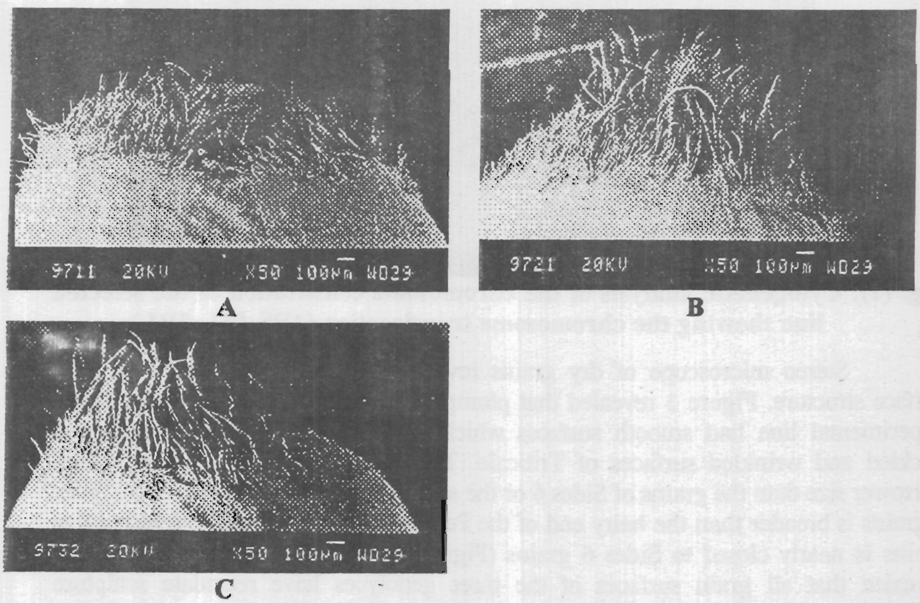

Fig. (4): Stereo microscope micrographs of the hairy end of the grains for the three genotypes. A: Sides 6, B. The selected line and C: Pc 62.

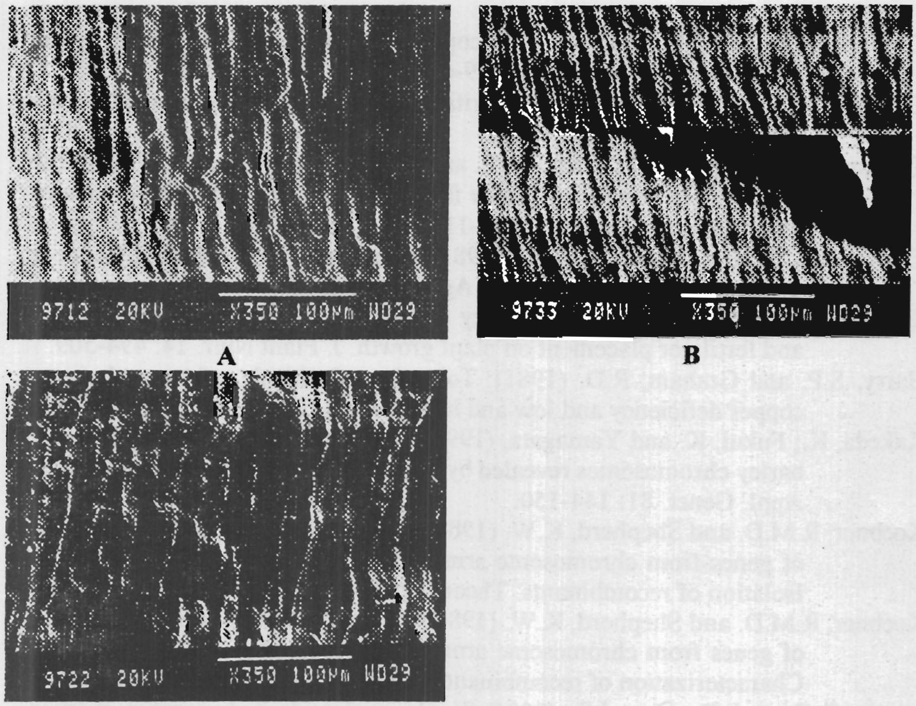

Fig. (5): Surface scan by electron microscopy in three wheat genotypes show the reticulate sculpture. A: Sides 6, B. The selected line and C: Pc 62.

REFERENCES

Barthlott, W. (1984): Microstructural features of seed surfaces. In (concept in plant taxonomy). (ed. V. H. Heywood and D. M. Moore), pp. 95, Academic Press, London.

Brown, J.C. and Clark, R.B. (1977): Copper is essential to wheat cultivar reproduction. Plant Soil, 48: 509-523.

Driscoll, C.J. and Sears, E.R. (1965): Mapping of a wheat-rye translocation. Genetics, 51: 439-443.

Gill, B.S.; Friebe, B. and Endo, T.R. (1991): Standard karyotype and nomenclature system for description of chromosome band and structural aberrations in wheat (*Triticum aestivum L.*) Genome. 34: 830-839.

Giraldez, R.; Cermeno, M.C. and Orelliana, J. (1979): C-banding pattern in the chromosomes of inbred lines and open-pollinating varieties of rye. Z. pflanzenzuchtg. 83: 40-48.

Graham, R.D. (1975): Male sterility in wheat plants deficient in copper. Nature. 254: 514-515.

Graham, R.D. (1976): Anomalous water relations in copper-deficient wheat plants. Aust. J. Exp. Bot., 3: 229-236.

Graham, R.D. (1981): Absorption of copper by wheat, rye and some hybrid genotypes. J. Plant Nut. 3: 679-686.

Graham, R.D. (1984): Breeding for nutritional characterization in cereals. Adv. Plant Nut. 1: 57-102.

Graham, R.D.; Ascher, J.S.; Ellis, P.A.E. and Shepherd, K.W. (1987): Transfer to wheat of the copper efficiency factors carried on rye chromosome arm 5RL. Plant and Soil., 99: 107-114.

Graham, R.D. and Nambiar, E.K. (1981): Advances in research in copper deficiency in cereals. Aust. J. Agric. Res. 32: 1009-1037.

Grundon, N.J. (1991): Copper deficiency of wheat: effect of soil water content and fertilizer placement on plant growth. J. Plant Nutr. 14: 494-509.

Harry, S.P. and Graham, R.D. (1981): Tolerance of triticale, wheat and rye to copper deficiency and low and high soil pH. J. Plant Nutr. 3: 721-730.

Kakeda, K.; Fukui, K. and Yamagata, (1991): Heterochromatic differentiation in barley chromosomes revealed by C- and N-banding techniques. Theor. Appl. Genet. 81: 144-150.

Koebner, R.M.D. and Shepherd, K.W. (1986a): Controlled introgression to wheat of genes from chromosome arm 1RS by induction of allosyndesis. 1. Isolation of recombinants. Theor. Appl. Genet., 73: 197-208.

Koebner, R.M.D. and Shepherd, K.W. (1986b): Controlled introgression to wheat of genes from chromosome arm 1RS by induction of allosyndesis. 2. Characterization of recombinants. Theor. Appl. Genet., 73: 209-217.

Leach, R.C. and Dundas, I.S. (2006):Single nucleotide polymorphic marker enabling rapid and early screening for the homoeolocus of β-amylase-R1: a gene linked to copper efficiency on 5RL. Theor. Appl. Genet. 113: 301-307.

Loutfy, A.E. (1992): The seed morphology of the malvaceae and its taxonomic value., Ph.D., Fac. Sci. Ain Shams Univ., Egypt. 253 pp.

Lukaszewski, A.J. and Gustafson, J.P. (1983): Translocations and modifications of chromosomes in triticale X wheat hybrids. Theor. Appl. Genet. 64: 239-248.

Owuoche, J.O.; Briggs, K.G.; Taylor, G.J. and Penney, D.C. (1994): Response of eight Canadian spring wheat (*Triticum aestivum L.*) cultivars to copper –pollen viability, grain yield, plant and yield components. Can. J. Plant Sci., 73: 25-30.

Riley, R.; Chapman, V. and Miller, T.E. (1970): An. Rep. Plant Breeding Int., Cambridge.

Salama, Hanan (2003): Taxonomic studies on the grains of some wild and cultivated taxa of poaceae. M. Sc. Thesis in Botany Fac. Agric.; Cairo Univ.

Schlegel,R.; Kynsat, R.; Schwazacher, T.; Rőmheld, V. and Walter, A. (1993): Mapping of genes for copper efficiency in rye and the relationship between copper and iron efficiency. Plant and Soil, 154: 61-65.

Schlegel, R.; Werner, T. and Hulgfnhof, F. (1991): Confirmation of a 4BL-5RL wheat –rye chromosome translocation line in the wheat cultivar "Viking cultivar" showing high copper efficiency. Plant breeding., 107: 226-234.

Sears, E.R. (1967): Induced transfer of hairy neck from rye to wheat. Z. Pflanzenzüchtung, 57: 4-25.
Soon, Y.K., Clayton, G.W. and Clarke, P.J. (1997): Content and uptake of phosphorous and copper by spring wheat effect of environment, genotype and management. J. Plant Nutr. 20: 925-937.
Sybenga, J. (1983): Rye chromosome nomenclature and homoeology relationships. Pflanzenzüchtung, 90: 297-304.

دراسات للتركيب الدقيق لسطح الحبوب وسيتولوجية فى سلالة قمح منتخبة تحتوى على انتقال بين الكروموسوم 4B والقطعة الكروموسومية 5Rl

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تمت دراسة بعض الصفات الاقتصادية فى كل من الابوين؛ سدس ٦ وبى سى ٦٢ بالاضافة الى السلالة المنتخبة كما تمت دراسة التركيب الدقيق للشكل الظاهرى لحبوب التراكيب الوراثية الثلاثة بإستخدام الميكروسكوب الالكترونى إضافة الى دراسة سيتولوجية لتحديد عدد الكروموسومات والقطعة الكروموسومية المنتقلة بإستخدام تكنيك الصبغ C-banding. أوضحت النتائج ان معظم الصفات الاقتصادية فى نسل السلالة المنتخبة كان متقاربا مع الصنف سدس ٦. بينما كان محصول السنبلة ٤,١٣ جم ووزن ال ١٠٠٠حبة ٤٨,٥٠ جم وهواقل مما فى حالة الصنف سدس ٦.

بالنسبة للتحليل الكروموسومى، بين الدور الاستوائى بإستخدام تكنيك الصبغ بالجيمسا C-banding أن عدد الكروموسومات فى السلالة المنتخبة هو ٢ن =٤٢ بالاضافة الى التعرف على كروماتين الراى. أظهر التكنيك الحزمى C-banding أن السلالة المنتخبة تحتوى على كروموسوم به انتقال ويشمل هذا الانتقال الذراع القصير والذراع الطويل للكروموسوم 4B بالاضافة الى الذراع الطويل للكروموسوم 5RL والذى يمكن يميزه بالحزم القوية الطرفية وتحت الطرفية.

بالنسبة للتركيب الدقيق لحبوب الابوين، سدس ٦ وبى سى ٦٢ بالاضافة الى السلالة المنتخبة كانت كل الحبوب ذات اسطح ملساء والتى يمكن تمييزها بسهولة عن الاسطح المجعدة فى حالة التريتيكال. أظهرت حبوب الصنف بى سى ٦٢ حجما ضيقا مقارنة بحبوب الصنف سدس ٦ والسلالة المنتخبة. كانت النهاية الزغبية لحبوب الصنف سدس ٦ عريضة اكثر مقارنة بالنهاية الزغبية للصنف بى سى ٦٢. كانت النهاية الزغبية لحبوب السلالة المنتخبة تقريبا مشابهه لحبوب الصنف سدس ٦. بين المسح الالكترونى الدقيق للسطح الخارجى للحبوب الشكل الشبكى فى التراكيب الوراثية الثلاثة. كما أظهر أن سطح الحبوب يكون شبكيا ضيقا اكثر بالمقارنة بسطح الحبوب للصنف سدس ٦ والسلالة المنتخبة. لم يكن هناك اختلافا واضحا بين سطح الحبوب فى الصنف سدس ٦ والسلالة المنتخبة.