MODIFICATIONS OF GENE EXPRESSION OF SOME QUALITY TRAITS IN BREAD WHEAT USING GAMMA IRRADIATION

HANAN M. MANSOUR\textsuperscript{1}, F. M. ABDEL-TAWAB\textsuperscript{2}, EMAN M. FAHMY\textsuperscript{2}, SUZAN R. MAHROUS\textsuperscript{1}, HALA F. EI\textsuperscript{1}SSA\textsuperscript{3} AND O. M. SALEH\textsuperscript{1,3}

1. National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt
2. Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
3. Agriculture Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt

Wheat is the most widely grown crop in over 120 countries worldwide (FAO, 2002).\emph{Triticum aestivum} (including the common bread wheat) is the most commercially relevant species of genus \emph{Triticum} widely grown all over the world. In addition to its high-energy content, wheat is also a good source of protein and contains considerably more protein on average than other cereals. The proteins of wheat are complex, they can be divided into two broad categories based on their biological function to the biologically active enzymes; albumins and globulins and the biologically inactive storage protein; gliadins and glutenins (Lookhart and Bean, 2000). The gliadins and glutenins are referred to collectively as the gluten proteins, and are mainly located within the mealy endosperm of the grain.

The gluten proteins play a key role in the formation of dough for bread making, and it was considered as important target to several investigations for the close correlation between the strength and quantity of gluten protein with the wheat bread making quality (Moczulski and Salmanowicz, 2003). Gamma ray has been used in a number of previous investigations as a good inducer for many useful mutations. Good results were obtained on the level of yield parameters such as number of tillers/plant, number of spikes/plant, grain yield per plant and weight of one hundred grains (Abdel-Hady and Ali, 2006; Irfaq and Nawab, 2001). On the other hand, many investigations studied the effect of radiation on wheat quality improvement to extend shelf life of whole-wheat flour (Marathe \textit{et al.}, 2002) and to increase the activity of wheat grain endogenous amylases (Gralik and Warchalewski, 2006). In addition, there are a number of studies to elucidate the effect of gamma irradiation treatments on gluten protein (Koksel \textit{et al.}, 1998) and gluten fractions (Nayeem \textit{et al.}, 1999).

The objectives of the present study were to:

1. Investigate the effects of gamma radiations on some yield-related-traits associated with to the quality of wheat grains in four Egyptian wheat cultivars.
2- Study the effect of different doses of gamma irradiation on some quality traits of wheat grains in these cultivars.

3- Study possible modification in gene expression of irradiated plants through SDS-protein fractions.

MATERIALS AND METHODS

1. Plant materials

Dry grains of four bread wheat cultivars; Sakha-92, Sakha-61, Sids-1 and Giza-168 were irradiated with three doses of gamma rays (100, 150 and 200 Gy in addition to 0 Gy as control). Irradiation treatments were achieved by a $^{60}$Co gamma unit which delivered 5.6 KGY per hour.

2. Methods

2.1. Field experiment

Irradiated and non-irradiated grains of the four cultivars were sown to obtain M$_1$ plants, and then M$_2$ and M$_3$ generations. Data on plant height (cm), number of tillers/plant, number of spikes/plant, length of main spike (cm), grain yield per plant (g), and weight of 100 grain (g) traits were taken on M$_2$ generation. The outstanding to the plant at number of tillers and number of spikes per plant followed by high in grain yield per plant were selected from each treatment to examine the sedimentation value as an indicator of gluten strength, the ratio of both gluten fractions; gliadin and glutenin and for SDS- electrophoresis (SDS-PAGE). The data of M$_1$, M$_2$ and M$_3$ generation were statistically analyzed for split plot design according to Gomez and Gomez (1984). The Duncan's test was used to estimate the differences between means (Waller and Duncan, 1969).

2.2. Quality experiments

Regarding gluten quality and determination of the SDS-sedimentation test (MST) values, the method of Dick and Quick (1983) was applied by using 1.0 g of ground wheat for the sample. The concentration and percentage of gliadin and glutenin for irradiated and non-irradiated samples were determined according to the method of Suchy et al. (2007).

2.3. SDS-electrophoresis

Gliadin and glutenin protein fractions were extracted according to the method of Ariyama and Khan (1990). Protein fractions were performed on vertical slabs gel electrophoresis apparatus according to the method of Laemmli (1970) modified by Studier (1973). Gels were photographed using a digital camera and scanned with Bio-Rad video densitometer model 620, at a wave length of 577. Software data analysis for Bio-Rad Model 620 USA densitometer and computer were used as according to the manufacturer's instructions.

RESULTS AND DISCUSSION

1. Effect of gamma irradiation on yield-related traits in M$_2$ generation at harvest

Twenty plants from each cultivar under each treatment were selected ran-
domly to measure the means of the six aforementioned yield-related traits (Data not shown). It was noted that the effect of \( \gamma \)-irradiation on the four wheat cultivars generally showed significant differences for the yield-related traits which agreed with Irfaq and Nawab (2001) and Khan et al. (2003). The cultivars showed different trends under the three doses for the number of tillers/plant and number of spikes/plant. Irradiated plants of Sakha-92 cultivar showed slight decrease for number of tillers/plant and number of spikes/plant at both 100 and 200 Gy doses, while it exhibited significant decrease at 150 Gy dose.

According to the importance of the yield-related traits as economic traits and in addition to their associations with the quality traits of both proteins and the grains of wheat (Huang et al., 2006), the outstanding plants in the number of tillers followed by increasing grain yield per plant were selected from each cultivar in each dose to assess the effect of the used gamma doses on the quality traits; sedimentation value and concentration of gluten fractions (gliadin and glutenin). In addition, to study the differences at the level of gene expression using SDS- gluten electrophoresis and compare the results with their respective controls. The values of the six studied yield-related traits for the superior selected plants (Data not shown) indicated that the irradiated plants of cultivar Sids-1 at 100, 150 and 200 Gy doses had the highest values for number of spikes/plant and number of tillers/plant and grain yield at 200, 100 and 150 Gy doses, respectively, compared with the other selected plants. Although Sids-1 under 200 Gy dose scored the same values of both number of tillers and spikes per plant compared with control, one outstanding individual plant was taken from this treatment which scored 71 tillers and spikes per plant as a good model for wheat quality studies.

2. Effect of gamma radiation on the quality of gluten and ratio of its fractions

The concentration and percentage of each gluten fractions; gliadin and glutenin and the SDS-sedimentation test (MST) values determined for the selected plants (parents) and \( M_2 \) plants for each treatment indicated differences in the determined values between the cultivars and among the irradiated sample and it’s control as shown in Table (1). The results illustrated that; the variety Sakha-92 showed the highest sedimentation value compared with other cultivars, and this was associated with the high gluten concentration. The irradiated lower samples for both Sakha 92 and Giza-168 cultivars at the three used doses recorded lower sedimentation value comparing with control. On the other hand, the sedimentation value was increased for the irradiated sample compared with control for both Sids-1 and Sakha-61 cultivars. It is noteworthy that the increasing in sedimentation value was associated with the increase of the glutenin concentration comparing with control in addition to the decrease in the gliadin concentration com-
paring with control. For Giza-168 cultivar, although the increase in the total protein in all irradiated sample comparing with control was observed, it showed a decrease in the sedimentation value in all three used doses. This could be due to the gluten strength affected by the ratio of both gliadin and glutenin that increasing of glutenin ratio led to increasing in the sedimentation value which means a more gluten strength. The increasing in gluten strength is not related only to the increasing in the total protein, but the difference in the ratio between the two fractions of gluten determined the gluten strength and the quality of end product until with the same concentration of the total protein. Our results agreed with those of Wieser et al. (2003) who found that both the amount of glutenin subunits (positively) and the ratio of gliadins to glutenin subunits (negatively) had a strong influence on the maximum resistance and extension area of gluten and on the bread volume.

3. Gliadin and glutenin protein electrophoresis

The results of SDS-PAGE analysis of wheat protein fractions of sample under study are illustrated in Figs (1 & 2) and Tables (2 & 3).

The results of electrophoretic analysis of gliadins showed a total of 21 bands ranging from 2.45 to 71.28 KDa which not necessarily present in all tested samples. The number of total bands varied between the four studied cultivars whereas the lowest number of 14 bands appeared in cultivar Sakha-92, and the highest number of 20 bands appeared in cultivar Sakha-61. The region of omega-gliadin had a wider range for the number of bands (eight bands) than all other regions of gliadins, these results agreed with those reported by Rashed et al. (2007). Among such gliadin protein bands, eight bands were commonly detected in all irradiated and non-irradiated wheat samples with different molecular weights (monomorphic bands). Three common bands at mobilities of 0.21, 0.23 and 0.39 with molecular weights 71.28, 67.29 and 30.70 KDa, respectively, were found in omega zone, five at mobility 0.73, 0.78, 0.85, and 0.95 with molecular weights 6.56, 5.19, 4.32, 3.66 and 2.45 KDa, respectively, were found in alpha zone. In addition, two bands were present in the control samples for the four wheat cultivars while they disappeared in some irradiated samples such as Sids-1 and Giza-168 cultivars, one of these bands in omega zone at mobility 0.37 with molecular weight of 34.50 KDa and the other in gamma zone at mobility of 0.41 with molecular weight of 28.17 KDa. The other bands were distributed between irradiated and non-irradiated samples (polymorphic bands). The band at mobility of 0.46 with molecular weight of 21.19 KDa identified Sakha-92, Sakha-61 and Sids-1 cultivars while it was absent from cultivar Giza-168 although it was detected in its three irradiated samples. The band at mobility of 0.25 with molecular weight of 58.15 KDa identified one sample only which was irradiated by 100Gy dose in the cultivar Sakha-61 while this band disappeared from all another samples. The band at mobility of 0.26 with molecular weight of 54.17 KDa
identified the sample of cultivar Sids-1 and the three irradiated samples for both Sakha-61 and Giza-168 cultivars and the irradiated sample at 100 Gy dose for cultivar Sakha-92. The band at mobility of 0.32 with molecular weight of 40.01 KDa identified only cultivar Sakha-61 control sample while it disappeared in the other cultivars, it was noted that it also appeared with the irradiated sample at 100 Gy dose for the four studied cultivars.

The band at mobility of 0.54 with molecular weight of 15.43 KDa was present in cultivars; Sakha-61 and Sids-1 control samples although it disappeared from some of their irradiated samples, while it was absent in both Sakha-92 and Giza-168 cultivar’s samples and all their irradiated samples at all used doses. The bands at mobility of 0.43 and 0.59 with molecular weights of 22.48 and 11.31 KDa, respectively, were present among both Sakha-92 and Sakha-61 cultivars and all their irradiated samples at the three used doses while neither was present in the control samples for Sids-1 and Giza-168 cultivars nor their irradiated samples. The band at mobility of 0.66 with molecular weight of 9.43 KDa was present in the three cultivars; Sakha-61, Sids-1 and Giza-168, although it was absent in Sakha-92 cultivar, it was detected in the irradiated sample at 100 Gy dose for Sakha-92 cultivar. The band at mobility of 0.69 with molecular weight of 7.79 KDa was exhibited only in the control sample for Sakha-61 cultivar and both its irradiated samples; 100 and 150 Gy doses.

For the glutenin analysis, 25 polypeptide bands were observed with molecular weights ranging from 11.59 to 113.09 KDa which were not necessarily present in all varieties. Among such glutenin protein’s bands, eleven bands were commonly detected in all studied samples with different molecular weights (monomorphic bands) while, another fourteen bands were distributed between the irradiated and non irradiated studied samples (polymorphic bands). The bands with molecular weight of 113.09 and 97.46 KDa were identified only in the irradiated sample at 100 Gy dose for cultivar Sakha-61. The band with molecular weight of 59.93 KDa was present in four cultivars in addition to all irradiated samples for cultivars; Sids-1 and Giza-168, while it was absent in the irradiated samples for cultivar Sakha-61 in all doses and the irradiated sample for cultivar Sakha-92 at 100 Gy dose. The bands with molecular weights of 87.98, 71.19 and 20.37 KDa were present only in the irradiated samples for Sids-1 cultivar at the three used doses. The band with molecular weight 65.40 KDa was present in irradiated samples of cultivar Sids-1 and the irradiated sample at 100 Gy dose for cultivar Giza-168. The band with molecular weight of 51.00 KDa was exhibited only in the irradiated samples for cultivar Giza-168 in the three used doses. The band with molecular weight of 43.87 KDa was present in the control sample of cultivar Sakha-92 and the irradiated samples for cultivar Sakha-61 while it was absent from the other three cultivars. The band with molecular weight of 27.77 KDa was present in Sakha-92 and Sakha-61 culti-
vars and their irradiated samples and all irradiated samples of cultivar Sids-1. The band with molecular weight 25.40 KDa was absent in the four studied cultivars while it was detected at a number of the irradiated samples of these cultivars.

4. The relationships between gliadin & glutenin bands and wheat quality

The relationship between the gliadin & glutenin bands for irradiated and non-irradiated samples with sedimentation values was investigated. At the level of SDS-electrophoresis pattern of gliadin, it was not possible to relate between the observed bands and the increase or decrease in sedimentation values. On the other hand, SDS-electrophoresis pattern of glutenin exhibited a number of bands that may be associated with low and high quality. Three bands number; 3, 7 and 20 with molecular weights; 87.98, 71.19 and 20.37 KDa, respectively, could be detected only in the three irradiated samples of Sids-1 cultivar, these samples scored increasing in the sedimentation values comparing with the control. This indicated that these three bands could be associated with the increase of gluten strength. It was worthy to mention that band 7 with molecular weight of 71.19 KDa was identified by Payne et al. (1979) who named it subunit 3 and they found that most of investigated plants which contained this band had low sedimentation values; they suggested that there is inverse correlation between subunit 3 and quality. At M3 generation, two plants from cultivar Sakha-92 at 100 Gy dose and one from cultivar Sk-61 at 200 Gy dose could be selected for superiority in grain yield per plant and sedimentation values, respectively, comparing with the control. Two superior plants at 100 and 150 Gy doses each, and three plants from 200 Gy dose were selected from M3 generation plants of cultivar Sids-1 due to their superiority in the six yield-related traits and sedimentation values comparing with the control. These selected plants in M3 generation of the three previous cultivars could be planted for next generations to assess the stability of the mutant genotypes.

SUMMARY

Four Egyptian bread wheat cultivars (Triticum aestivum L.); Sakha-92, Sakha-61, Sids-1 and Giza-168 were exposed to different gamma radiation doses; 0, 100, 150 and 200 Gy, from a 60Co gamma ray source. The effect of gamma rays on a number of yield-related traits and sedimentation values were studied on M1, M2 and M3 generations. The effect of gamma rays at 150 Gy on Sids-1 cultivar gave significant increase and surpassed the control in all yield-related traits in M3. The increase or decrease in sedimentation values were more related to the variation
in glutenin concentration. The difference in the gliadin to glutenin ratio was more than the variation in gliadin concentration. Giza-168 cultivar - under the three doses - recorded low mean values for all traits comparing with the control. Three SDS-bands with molecular weights of 87.98, 71.19 and 20.37 KDa could be used as indication related to the increase of gluten strength in the three irradiated samples of Sids-1 cultivar which scored marked increase in sedimentation values comparing with control. On the contrary, band with molecular weight; 51.00 KDa identified only the three irradiated samples of Giza-168 cultivar (with low sedimentation value), this may be considered as marker for low quality gluten. Three M$_3$ generation plants in Sids-1 cultivar from different irradiation doses showed outstanding performance for both the six yield-related traits and sedimentation values comparing with the M$_3$ control plant.

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251.
Table (1): The effect of γ-irradiation on the sedimentation value (MST) and the concentration of gliadin and glutenin proteins in the four wheat cultivars (P) and their M₂.

| Genotypes | treatment | Total concentration | Gliadin concentration | Glutenin concentration | Gliadin% | Glutenine% | MST-test value |
|-----------|-----------|---------------------|-----------------------|------------------------|----------|-----------|----------------|
| Sakha92 (P) | Control  | 0.518               | 0.419                 | 0.099                  | 80.97    | 19.03     | 61.7           |
| Sakha92   | 100 Gy    | 0.523               | 0.421                 | 0.102                  | 80.56    | 19.44     | 60.3           |
| Sakha92   | 150 Gy    | 0.517               | 0.422                 | 0.095                  | 81.65    | 18.35     | 59.0           |
| Sakha92   | 200 Gy    | 0.515               | 0.431                 | 0.084                  | 83.80    | 16.20     | 55.4           |
| Sakha61 (P) | Control  | 0.485               | 0.378                 | 0.103                  | 78.76    | 21.07     | 50.0           |
| Sakha61   | 100 Gy    | 0.515               | 0.383                 | 0.133                  | 74.27    | 25.73     | 64.0           |
| Sakha61   | 150 Gy    | 0.564               | 0.406                 | 0.158                  | 71.92    | 28.08     | 72.7           |
| Sakha61   | 200 Gy    | 0.564               | 0.441                 | 0.122                  | 78.29    | 21.71     | 58.0           |
| Sids1     | Control  | 0.596               | 0.489                 | 0.108                  | 81.94    | 18.06     | 60.0           |
| Sids1     | 100 Gy    | 0.563               | 0.436                 | 0.127                  | 77.49    | 22.50     | 70.3           |
| Sids1     | 150 Gy    | 0.632               | 0.452                 | 0.177                  | 71.52    | 28.25     | 80.3           |
| Sids1     | 200 Gy    | 0.634               | 0.451                 | 0.183                  | 70.44    | 29.56     | 79.3           |
| Giza168   | Control  | 0.490               | 0.384                 | 0.106                  | 78.41    | 21.59     | 60.3           |
| Giza168   | 100 Gy    | 0.570               | 0.474                 | 0.095                  | 83.25    | 16.75     | 51.3           |
| Giza168   | 150 Gy    | 0.594               | 0.526                 | 0.068                  | 86.33    | 13.67     | 43.3           |
| Giza168   | 200 Gy    | 0.550               | 0.459                 | 0.090                  | 83.63    | 16.37     | 45.3           |
Table (2): SDS-PAGE pattern of alcohol soluble protein (gliadin) from Sakha-92, Sakha-61, Sids-1 and Giza-168 cultivars under γ-irradiation.

| BN   | MW  | RF   | Sk92 control | Sk92 100 Gy | Sk92 150 Gy | Sk92 200 Gy | Sk61 control | Sk61 100 Gy | Sk61 150 Gy | Sk61 200 Gy | Sids-1 control | Sids-1 100 Gy | Sids-1 150 Gy | Sids-1 200 Gy | Giza168 control | Giza168 100 Gy | Giza168 150 Gy | Giza168 200 Gy |
|------|-----|------|--------------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|---------------|--------------|--------------|--------------|----------------|----------------|----------------|----------------|
| ozone 1 | 71.28 | 0.21 | ++++       | ++++        | ++++        | ++++        | ++++         | ++++        | ++++        | ++++        | ++++          | ++++         | ++++         | ++++         | ++++           | ++++           | ++++           | ++++           |
| 2  | 67.29 | 0.22 | ++++       | ++++        | ++++        | ++++        | ++++         | ++++        | ++++        | ++++        | ++++          | ++++         | ++++         | ++++         | ++++           | ++++           | ++++           | ++++           |
| 3  | 58.15 | 0.25 | -           | -           | -           | ++          | -            | -           | -           | -           | ++            | ++           | ++           | ++           | ++             | ++             | ++             | ++             |
| 4  | 54.17 | 0.26 | +           | -           | -           | ++          | ++          | ++          | ++          | ++          | -             | -           | -            | ++           | ++             | ++             | ++             | ++             |
| 5  | 47.08 | 0.29 | -           | +++         | -           | ++          | -           | +           | ++          | -           | +++          | ++           | ++          | ++           | ++             | ++             | ++             | ++             |
| 6  | 40.01 | 0.32 | -           | ++          | -           | ++          | -            | -           | -           | -           | ++            | +           | -            | ++           | ++             | ++             | ++             | ++             |
| 7  | 34.5  | 0.37 | +++         | +++         | ++          | ++          | +++         | +++         | +++         | ++          | +++          | ++           | ++          | ++           | ++             | ++             | ++             | ++             |
| 8  | 30.7  | 0.39 | ++++        | ++++        | +++         | +++         | ++++        | ++++        | ++++        | ++++        | -            | +++         | +++          | +++          | ++             | +++             | +++             | +++             |
| γzone 11 | 28.17 | 0.41 | +++         | +           | +++         | +++         | +++         | +++         | +++         | +++         | -            | +++         | +++          | +++          | ++             | +++             | +++             | +++             |
| 10 | 22.48 | 0.43 | +           | ++          | +           | +          | +           | +           | +           | -           | -            | -           | -            | -            | -              | -               | -               | -               |
| 11 | 21.9  | 0.47 | ++          | -           | ++         | ++          | ++          | ++         | ++          | ++          | +            | ++          | -           | -            | +++            | +               | +++             | ++             |
| 12 | 15.43 | 0.54 | -           | -           | -           | +          | +           | -           | +           | -           | +            | -           | +            | +            | +++             | +               | +++             | ++             |
| 13 | 11.31 | 0.59 | +++        | +++         | +++         | +++         | +++        | +++        | +++        | +++        | -            | -           | +            | -            | -              | -               | -               | -               |
| Bzone 14 | 10.61 | 0.63 | +++         | +++         | +++         | +++         | +++         | +++        | +++        | +++        | -            | ++         | ++          | ++          | ++             | ++             | ++             | ++             |
| 15 | 9.43  | 0.66 | -           | -           | -           | +          | +           | -           | -           | ++         | ++          | ++          | ++          | ++          | ++             | ++             | ++             | ++             |
| 16 | 7.79  | 0.69 | -           | -           | -           | +          | +           | -           | -           | -           | -            | -           | -            | -            | -              | -               | -               | -               |
| azon 17 | 6.56  | 0.73 | +++         | +++         | +++         | +++         | +++        | +++        | +++        | +++        | +++          | +++         | +++          | +++          | +++             | +++             | +++             | +++             |
| 18 | 5.19  | 0.79 | +++         | +++         | +++         | +++         | +++        | +++        | +++        | +++        | +++          | +++         | +++          | +++          | +++             | +++             | +++             | +++             |
| 19 | 4.32  | 0.84 | +++         | +++         | +++         | +++         | +++        | +++        | +++        | +++        | +++          | +++         | +++          | +++          | +++             | +++             | +++             | +++             |
| 20 | 3.66  | 0.89 | +++         | +++         | +++         | +++         | +++        | +++        | +++        | +++        | +++          | +++         | +++          | +++          | +++             | +++             | +++             | +++             |
| 21 | 2.45  | 0.95 | +++         | +++         | +++         | +++         | +++        | +++        | +++        | +++        | +++          | +++         | +++          | +++          | +++             | +++             | +++             | +++             |

Total of band 14 17 14 19 20 18 16 15 12 16 15 13 16 15 14

Bands were indicated by dye marker intensities; (+): very weak, (++): weak, (+++): intensive, (++++): very intensive

RF=Relative front, MW=Molecular weight, BN=band number
Table (3): SDS-PAGE pattern of acetic acid soluble proteins (glutenin) from Sakha-92, Sakha-61, Sids-1 and Giza-168 cultivars under γ-irradiation.

| BN | MW  | RF  | SK92 control | SK92 100 Gy | SK92 150 Gy | SK92 200 Gy | SK61 control | SK61 100 Gy | SK61 150 Gy | SK61 200 Gy | Sids1 control | Sids1 100 Gy | Sids1 150 Gy | Sids1 200 Gy | Giza168 control | Giza168 100 Gy | Giza168 150 Gy | Giza168 200 Gy |
|----|-----|-----|--------------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|---------------|--------------|--------------|--------------|
| 1  | 113.09 | 0.23 | -            | -           | -           | -           | +++          | -           | -           | -           | -            | -           | -           | -           | -             | -             | -             | -             |
| 2  | 97.46  | 0.28 | -            | -           | -           | -           | +++          | -           | -           | -           | -            | -           | -           | -           | -             | -             | -             | -             |
| 3  | 87.98  | 0.30 | -            | -           | -           | -           | -            | -           | -           | +           | +            | +           | +           | -           | -             | -             | -             | -             |
| 4  | 78.89  | 0.34 | -            | -           | -           | -           | -            | -           | -           | +           | +++          | +++         | ++          | +           | +++           | +++           | ++            | +             |
| 5  | 75.87  | 0.36 | +++          | ++          | +++         | ++          | +++          | +++          | +++         | +++         | +++          | +++         | ++          | +++         | +             | +++           | ++            | ++            |
| 6  | 73.78  | 0.37 | +++          | +++         | +++         | +++         | +++          | +++         | +++         | +++         | +++          | +++         | ++          | +++         | ++            | +++           | +++           | +++           |
| 7  | 71.19  | 0.39 | -            | -           | -           | -           | -            | -           | -           | -           | +++          | +++         | ++          | ++          | ++            | +++           | +++           | +++           |
| 8  | 65.40  | 0.42 | -            | -           | -           | -           | -            | -           | -           | +           | ++          | ++         | +           | ++         | ++            | +++           | ++           | +             |
| 9  | 59.93  | 0.44 | ++           | -           | +           | +           | -            | -           | +           | +++         | ++          | +++        | ++          | ++         | ++            | +++           | +++           | ++            |
| 10 | 51.00  | 0.48 | -            | -           | -           | -           | -            | -           | -           | -           | +++          | +++         | ++          | ++         | ++            | +++           | +++           | +++           |
| 11 | 48.07  | 0.51 | +++          | +++         | +++         | +++         | +++          | +++         | +++         | +++         | +++          | ++         | +          | ++         | ++            | +++           | +++           | +++           |
| 12 | 46.07  | 0.52 | -            | ++          | +++         | +++         | +++          | +++         | +++         | +++         | +++          | ++         | ++         | ++         | +++            | +++           | +++           | +++           |
| 13 | 43.87  | 0.62 | ++           | -           | -           | +++         | +++          | +++         | +++         | +++         | +++          | ++         | ++         | ++         | +++            | +++           | +++           | ++            |
| 14 | 32.83  | 0.68 | ++           | +           | +           | +           | +++          | +++         | +++         | +++         | +++          | ++         | ++         | ++         | +++            | +++           | +++           | ++            |
| 15 | 27.77  | 0.69 | ++           | ++          | +           | +           | +++          | +++         | +++         | +++         | +++          | ++         | ++         | ++         | +++            | +++           | +++           | ++            |
| 16 | 25.39  | 0.72 | ++           | +           | ++          | ++          | +++          | +++         | +++         | +++         | +++          | ++         | ++         | ++         | +++            | +++           | +++           | ++            |
| 17 | 24.43  | 0.75 | +++          | +++         | +++         | +++         | +++          | +++         | +++         | +++         | +++          | +++        | ++         | ++         | +++            | +++           | +++           | ++            |
| 18 | 23.27  | 0.76 | ++           | -           | ++          | ++          | ++           | ++          | ++         | +           | +++         | +          | ++         | ++         | +++            | +++           | +++           | +++           |
| 19 | 21.9   | 0.77 | +++          | +++         | +++         | +++         | +++          | +++         | +++         | +++         | +++          | +++        | ++         | ++         | +++            | +++           | +++           | +++           |
| 20 | 20.37  | 0.79 | -            | -           | -           | -           | -            | -           | +++         | +++         | +++          | +++        | ++         | ++         | +++            | +++           | +++           | +++           |
| 21 | 17.73  | 0.84 | ++           | ++          | ++          | ++          | ++           | ++          | ++         | ++          | ++          | +++        | ++         | ++         | +++            | +++           | +++           | +++           |
| 22 | 15.06  | 0.89 | ++           | ++          | ++          | ++          | ++           | ++          | ++         | ++          | ++          | ++         | +++        | ++         | +++            | +++           | +++           | +++           |
| 23 | 14.79  | 0.92 | +++          | +++         | +++         | +++         | +++          | +++         | +++         | +++         | +++          | +++        | +++        | +++        | +++            | +++           | +++           | +++           |
| 24 | 12.68  | 0.97 | +++          | +++         | +++         | +++         | +++          | +++         | +++         | +++         | +++          | +++        | +++        | +++        | +++            | +++           | +++           | +++           |
| 25 | 11.52  | 0.98 | +++          | +++         | +++         | +++         | +++          | +++         | +++         | +++         | +++          | +++        | +++        | +++        | +++            | +++           | +++           | +++           |

Bands were indicated by dye marker intensities; (+): very weak, (++): weak, (+++): intensive, (++++): very intensive.
RF=Relative front, MW=Molecular weight, BN=band number.
Fig. (1): SDS-PAGE pattern of gliadin proteins in M₂ of the four wheat cultivars under different γ-irradiation doses.
(M= marker as KDa), 1; Sk92 (0 Gy), 2; Sk92 (100 Gy), 3; Sk92 (150 Gy), 4; Sk92 (200 Gy), 5; Sk61 (0 Gy), 6; Sk61 (100 Gy), 7; Sk61 (150 Gy), 8; Sk61 (200 Gy), 9; Sids1 (0 Gy), 10; Sids1 (100 Gy), 11; Sids1 (150 Gy), 12; Sids1 (200 Gy), 13; G168 (0 Gy), 14; G168 (100 Gy), 15; G168 (150 Gy), 16; G168 (200 Gy)

Fig. (2): SDS-PAGE pattern of glutenin proteins in M₂ of the four wheat cultivars under different γ-irradiation doses.
(M= marker as KDa), 1; Sk92 (0 Gy), 2; Sk92 (100 Gy), 3; Sk92 (150 Gy), 4; Sk92 (200 Gy), 5; Sk61 (0 Gy), 6; Sk61 (100 Gy), 7; Sk61 (150 Gy), 8; Sk61 (200 Gy), 9; Sids1 (0 Gy), 10; Sids1 (100 Gy), 11; Sids1 (150 Gy), 12; Sids1 (200 Gy), 13; G168 (0 Gy), 14; G168 (100 Gy), 15; G168 (150 Gy), 16; G168 (200 Gy)
KARYOTYPE CHARACTERIZATION OF ALFALFA COLLECTED FROM WESTERN DESERT OF EGYPT

R. M. KHALAF AND ABEER ELHALWAGI

National Gene Bank and Genetic Resources. Agricultural Research Center

Alfalfa (*Medicago sativa* L.) is widely planted in the Egyptian Western Desert Oases as forage and is most often harvest for hay, but can also be made into silage, grazed, or fed green. Alfalfa has the highest feeding value among common hay crops, being used less frequently as pasture. One of the major forage crops in the oases is alfalfa. Apart from being an important forage crop, it has also medicinal uses. It is a perennial crop that resides in the soil from three to twelve years, depending on the variety and climate. Alfalfa has the ability to fix nitrogen, one feddan of alfalfa adds about go to 100 Kg of atmospheric nitrogen to the soil year, which would benefit other crops.

Cytogenetical research on alfalfa lagged far behind other crops mainly because, alfalfa chromosomes are very small, the chromosomes are morphologically very similar, cultivated alfalfa has relatively high number of chromosomes (2n= 4X= 32), and alfalfa is an autotetraploid (Bauchan and Hussain, 1997).

Bolton (1962) described difficulties involved in the mitotic study of *Medicago sativa* due to its small chromosomes and chromosome stickiness however, other authors believe that *Medicago sativa* meiosis can be studied successfully (Clement and Leman, 1962). The first karyotype was published by Buss and Cleveland (1968) and additional karyotypes have been developed by Agarwal and Gupta (1983) and Bauchan and Campbell (1994).

The objectives of this study were to determine the chromosome numbers karyotype and ideograms for 20 accessions of alfalfa. In this investigation an attempt was made to study Karyotype characterization will indicate the following:

1- Determining area of chromosome.
2- Determining length of chromosome.
3- Evaluation of long arm and short arm chromosome.
4- Determining Centromeric index and Centromeric position of chromosomes for the landraces alfalfa.

MATERIALS AND METHODS

The present study has been carried out on twenty accessions chosen for cytogenetically studies. These accessions were collected from the Western Desert Oases in Egypt. The name and pedigree of these genotypes are presented in Table (1).
Cytological analysis

Chromosomal studies are based on visible characteristics of the chromosomes. Karyotype analysis is a well established method. It is based on the morphological characteristics of chromosomes and is widely used in cytogenetic analysis (Fukui and Kakeda, 1994). Imaging by digital camera in the C-metaphase of dividing root tips cells, pretreated with 0.05% colchicine and analyzed using the video test karyotype software. Measurement of the total length of chromosome (µm), long arm, short arm of chromosome, area of chromosome (µm), arm ratio, Centromeric position and Centromeric index percentage (length of short arm/length of chromosome ) were taken for every chromosome.

From the karyotype analysis of alfalfa genome, the four homologues (a, b, c and d) of each chromosome pair were judged according to similarities in length of short arm, long arm, total lengths, arm ratio, area and Centromeric index percentage. An average length, arm ratio, and Centromeric index percentage were calculated (a, b, c and d)/4 for each pair was determined and the chromosome pairs were arranged in descending order and were given the numbers from 1 to 8.

Samples preparation

Seeds were germinated on moisture filter paper in Petri dishes at 25 - 30°C in an incubator. Root tips were obtained 3 days after germination was initiated. The lateral roots were collected of about 1.5:2.0 cm length.

Colchicine treatment

The roots of 1-1.5 cm were placed in glass vials containing 2 ml of 0.05% colchicine for three hours at room temperature or ice water over night.

Fixation and slide preparation

1- Fixation was done using ethanol – glacial acetic acid (3:1 v/v) fixative. Then, samples were washed thoroughly with water.
2- Flamed by forceps and staining by the aceto orcein solution.
3- These stained samples were used for automatic scanning experiments.
4- Karyotype analysis was carried out using Image Process Analysis System (Video Test-Karyo). (Fukui and Kakeda, 1994)
5- The mean measurements in the c-metaphase of fifteen cells for each accession were used to construct the karyotype.

RESULTS AND DISCUSSION

Investigate the karyotype analysis of alfalfa that can be utilized different breeding programs. Karyotype analysis showed that all of studied accessions were autotetraploid, with a chromosome number of 2n= 4x= 32, Fig. (1a, b, c and d). Karyotype analysis includes area of chromosome, length of chromosome, arm ratio
and centromeric index, as well as centromeric position of all accessions are recorded (Tables 3, 4, 5 and 6) and illustrated in Fig. (1a, b, c and d).

1- Mean Karyotype of the area chromosome at the twenty landraces

The data obtained from the karyotype, expressed by area of chromosome in the twenty accessions are given in Table (2). This table showed that the variation in chromosome area among the different twenty studied accessions is observable. This variation ranged from the highest value of chromosome area in accession 7 (6.68 µm) to its lowest value (5.07 µm) in accession 3. Table (2) showed that all accessions have higher value of area chromosome than the accession 3. The maximum mean chromosome area was recorded in chromosome 1 of all accessions, while the minimum was recorded in chromosome 7 of the all accessions. The same result was recorded by Bauchan and Hossain (2001). The high level of chromosome area indicate that more DNA in the cells.

Identification of alfalfa chromosomes at pachytene has been conducted by Gillius (1970) and Ho and Kasha (1974). Using a computerized image analysis system it is possible to determine the homologous chromosomes and develop a karyotype based on chromosome arm length, arm ratio and total chromosome length (Bauchan and Campbell, 1994).

2- Mean karyotype of the length chromosome at twenty landraces

Figure (1a, b, c and d) and Table (3) illustrated the C-metaphase, karyotype and karyotype analysis for accessions. The data presented in table (3) showed that the chromosome length depended on twenty accessions. These differences ranged from the highest score of chromosome length in accession 8 (5.81 µm) to the lowest estimate in accession 3 (4.12 µm). However, all accessions exhibited highly frequencies of chromosome length than accession 3. The data of this table showed that differences in chromosome length could be attributed to the effects of environmental variation on studied strains. The maximum mean chromosome length was 8.55 µm recorded in chromosome 1 of accession 10, whereas the minimum was 3.07 µm recorded in chromosome 7 of accessions 3. The high level of chromosome length indicated that more crossing over. (Fayed et al., 1984)

Measuring chromosome from photomicrographs, Falistocco (1987) obtained much larger chromosome measurements than what have reported for the gene Medicago, for example, a total length ranging between 9 and 12 µm. Schlarbaum et al. (1988) karyotype tetraploid alfalfa from plants that had been regenerated from a single cell protoplast in tissue culture; however, it is known that plants regenerated from a tissue culture system potentially can have chromosomes which have been altered (Nagarajan and Walton, 1987).
3- Mean karyotype of the long arm and short arm chromosome at twenty accessions

The mean karyotype of the long arm and short arm chromosome the twenty different accessions are given in Tables (4 and 5). The accessions varied in their chromosome long arm and short arm, showing different trends. The highest chromosome long arm and short arm was exhibited by the accession 8 while the Lowes on was displayed in accession 3. The maximum mean chromosome long arm and short arm were 5.06 µm and 3.49 µm, respectively, recorded in chromosome 1 of accession 10, while the minimum chromosome were 1.58 µm and 1.48 µm recorded in chromosomes 7 of accession 3, respectively.

Preliminary studies of six accessions of tetraploid *ssp. falcata* offer a surprising result. Most of the plants possess chromosomes which have C-bands in addition to the normal Centromeric bands. There is a range from an accession which contains the fewest number of additional bands, 4 pairs of chromosomes have an extra telomeric band on their short arms, whereas the rest of the chromosomes only have a centromeric band, two accessions which have multiple bands on each chromosome and a banding pattern similar to doubled diploid *ssp. coerulea*. In general a majority of the heterochromatic bands appear in the short arms of the chromosomes with only chromosomes possessing interstitial bands on their long arms (Bauchan and Hossain, 2001).

4- Mean karyotype of the arm ratio, Centromeric index and Centromeric position at twenty accessions

The data of mean karyotype, expressed by the arm ratio, Centromeric index and Centromeric position in twenty accessions are given in Table (6). This table showed that the variation in arm ratio between the studied strains, this variation ranged from the highest score of arm ratio in accession 17 (1.96 µm) to its Lowes estimate (0.99 µm) in accession 11. However all accessions exhibited higher frequencies of arm ratio than accession 11. The maximum mean Centromeric index was 50.14% recorded in chromosome 5 of accession 11, while the minimum was 33.77% recorded in chromosome 2 of accession 7. The centromeric position ranged between submetacentric and metacentric in all accessions. The same results recorded by (Bauchan and Campbell, 1994).

The somatic chromosome karyotype of this subspecies consists of one pair of satellite chromosome (chromosome 8), four pairs of sub metacentric chromosomes (chromosomes 1-4), and there pairs of short metacentric chromosomes (chromosomes 5-7). Chromosome 8 possesses the nucleolar organizer region (NOR) of the alfalfa genome. The satellite chromosomes are diagnostic feature of the karyotype. The large submetacentric chromosome pair 1 is easy to distinguish if the chromosomes have not spiralized too much during pretreatment. Occasionally a tertiary constriction can be found on
chromosome 4 (Bauchan and Campbell, 1994).

The karyotype of African population alfalfa germplasm consists of one set of chromosomes with satellites (chromosome 8), for sets of submetacentric chromosomes (chromosome 1-4), and three sets of metacentric chromosomes (chromosomes 5-7). All of the chromosomes have Centromeric bands and a terminal band on the short arm, with the exception of the satellites (Bauchan and Hossain, 2001). Measurement of the total lengths of chromosome (µm), area of chromosome (µm²/µm) and Centromeric index percentage (length of short arm/length of chromosome) were taken for every chromosome based on Hussein (2005).

SUMMARY

In this investigation an attempt was made to study karyotype characterization of alfalfa. Another aim of this study was the determination of area chromosome, length chromosome, short and long arm chromosome, Centromeric index, and Centromeric position using twenty accessions. The studied accessions exhibited variation ranged from the highest score of area chromosome in accession 7 to its lowest estimate only in accession 3. The results exhibited differences ranging from the highest score of length chromosome in the accession 8 (5.81 µm) to the lowest in the accession 3 (4.12 µm). The Karyotype of accessions alfalfa consists of one set of chromosome with satellites (chromosome 8), four sets of submetacentric chromosomes (chromosome 1-4), and four sets of metacentric chromosomes (chromosomes 5-8). All of the landraces have the same number of chromosome 32 chromosome (2n= 4X= 32) with four nearly identical sets of chromosomes. Investigation the chromosomal maps of landraces that can be utilized in different breeding programs.

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Table (1): The names and pedigree of the twenty alfalfa accessions.

| Code | Accession Number |
|------|------------------|
| 1    | K 1              |
| 2    | K 2              |
| 3    | K 4              |
| 4    | K 14             |
| 5    | K 15             |
| 6    | D 20             |
| 7    | D 21             |
| 8    | D 22             |
| 9    | D 23             |
| 10   | D 25             |
| 11   | F 14             |
| 12   | F 15             |
| 13   | F 16             |
| 14   | F 17             |
| 15   | F 18             |
| 16   | S 1              |
| 17   | S 2              |
| 18   | S 4              |
| 19   | S 5              |
| 20   | S 6              |

| Pedigree                        | Origin  |
|---------------------------------|---------|
| Collection from El-Kharga       | Egypt   |
| Collection from El-Dakhla       | Egypt   |
| Collection from El-Farafra      | Egypt   |
| Collection from Siwa            | Egypt   |

Table (2): Mean of chromosome area of the eight chromosomes for accessions alfalfa.

| Code | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | Total area | Mean area |
|------|-----|-----|-----|-----|-----|-----|-----|-----|------------|-----------|
| 1    | 9.75| 7.59| 6.67| 6.03| 5.51| 4.82| 3.78| 5.45| 49.60      | 6.20      |
| 2    | 8.58| 7.50| 6.79| 5.59| 4.66| 4.01| 3.31| 5.53| 45.97      | 5.75      |
| 3    | 8.03| 6.02| 5.38| 5.02| 4.99| 3.65| 2.97| 4.55| 40.52      | 5.07      |
| 4    | 9.37| 8.27| 6.98| 6.38| 5.45| 4.40| 3.47| 5.20| 49.52      | 6.19      |
| 5    | 9.23| 8.33| 7.16| 6.09| 5.13| 4.25| 3.53| 5.07| 48.79      | 6.10      |
| 6    | 9.36| 7.70| 5.58| 5.35| 4.89| 4.25| 3.44| 6.30| 46.88      | 5.86      |
| 7    | 9.38| 8.28| 7.29| 6.61| 5.96| 5.24| 4.37| 6.28| 53.40      | 6.68      |
| 8    | 9.07| 7.89| 6.86| 5.86| 4.65| 3.80| 3.56| 5.61| 47.28      | 5.91      |
| 9    | 8.79| 7.76| 6.69| 5.69| 5.19| 4.15| 3.30| 5.41| 46.98      | 5.87      |
| 10   | 8.17| 7.19| 6.78| 5.64| 5.14| 4.49| 3.48| 5.81| 46.68      | 5.84      |
| 11   | 8.02| 6.48| 5.73| 5.49| 5.09| 4.53| 4.05| 7.46| 46.85      | 5.86      |
| 12   | 8.75| 8.02| 6.48| 5.97| 5.57| 5.11| 4.36| 7.19| 51.45      | 6.43      |
| 13   | 8.89| 7.94| 6.88| 6.25| 5.28| 4.65| 4.10| 7.12| 51.11      | 6.39      |
| 14   | 7.95| 6.62| 6.06| 5.77| 5.34| 4.34| 3.37| 6.63| 46.07      | 5.76      |
| 15   | 8.03| 6.85| 5.77| 5.21| 4.61| 4.18| 3.63| 6.20| 44.48      | 5.56      |
| 16   | 9.09| 8.16| 7.13| 6.25| 5.60| 5.19| 4.64| 6.77| 52.83      | 6.60      |
| 17   | 8.94| 7.66| 6.71| 5.79| 4.79| 4.02| 3.32| 4.96| 46.19      | 5.77      |
| 18   | 7.45| 6.34| 5.73| 5.20| 4.64| 4.07| 3.35| 6.09| 42.86      | 5.36      |
| 19   | 8.52| 6.79| 6.10| 5.48| 4.89| 4.28| 3.48| 5.44| 44.99      | 5.62      |
| 20   | 7.69| 6.55| 6.50| 4.86| 4.25| 3.64| 3.26| 5.87| 42.62      | 5.33      |
Table (3): Mean of chromosome length of the eight chromosomes for accessions alfalfa.

| Code | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | Total length | Mean length |
|------|------|------|------|------|------|------|------|------|--------------|-------------|
| 1    | 8.13 | 6.28 | 5.53 | 5.11 | 4.70 | 4.13 | 3.38 | 5.88 | 43.12        | 5.39        |
| 2    | 7.30 | 6.54 | 5.84 | 5.23 | 4.76 | 4.01 | 3.42 | 4.63 | 41.72        | 5.22        |
| 3    | 5.55 | 4.72 | 4.56 | 3.45 | 3.44 | 3.13 | 3.07 | 5.06 | 32.98        | 4.12        |
| 4    | 5.82 | 5.06 | 4.76 | 4.48 | 4.33 | 4.10 | 3.51 | 4.70 | 36.76        | 4.59        |
| 5    | 6.21 | 5.30 | 5.02 | 4.53 | 4.22 | 4.03 | 3.65 | 4.48 | 37.44        | 4.68        |
| 6    | 7.31 | 6.24 | 5.36 | 4.68 | 4.30 | 3.97 | 3.24 | 6.26 | 41.36        | 5.17        |
| 7    | 7.09 | 6.09 | 5.61 | 5.27 | 4.74 | 4.21 | 3.68 | 4.86 | 41.55        | 5.19        |
| 8    | 8.53 | 7.76 | 6.69 | 5.64 | 5.14 | 4.13 | 3.32 | 5.30 | 46.52        | 5.81        |
| 9    | 7.16 | 6.02 | 5.60 | 4.88 | 4.60 | 4.17 | 3.81 | 5.33 | 41.58        | 5.20        |
| 10   | 8.55 | 6.92 | 5.62 | 4.79 | 5.28 | 3.83 | 3.42 | 4.70 | 43.10        | 5.39        |
| 11   | 6.80 | 6.24 | 5.27 | 4.61 | 4.57 | 4.08 | 4.02 | 6.72 | 42.31        | 5.29        |
| 12   | 5.76 | 5.13 | 4.53 | 4.28 | 4.05 | 3.86 | 3.29 | 4.85 | 35.75        | 4.47        |
| 13   | 7.10 | 6.26 | 5.20 | 4.90 | 4.62 | 4.31 | 3.52 | 5.51 | 41.41        | 5.18        |
| 14   | 6.08 | 5.54 | 4.68 | 4.38 | 4.13 | 3.81 | 3.28 | 5.08 | 36.98        | 4.62        |
| 15   | 6.41 | 5.63 | 4.98 | 4.77 | 4.50 | 4.05 | 3.54 | 5.27 | 39.13        | 4.62        |
| 16   | 6.45 | 5.92 | 5.00 | 4.64 | 4.33 | 3.95 | 3.41 | 5.67 | 39.36        | 4.92        |
| 17   | 5.78 | 5.02 | 4.55 | 4.15 | 3.85 | 3.68 | 3.27 | 5.86 | 36.17        | 4.52        |
| 18   | 5.70 | 5.06 | 4.47 | 4.03 | 3.77 | 3.48 | 3.19 | 5.27 | 34.97        | 4.37        |
| 19   | 6.58 | 5.87 | 5.32 | 4.87 | 4.48 | 4.12 | 3.40 | 5.94 | 40.57        | 5.07        |
| 20   | 6.55 | 5.58 | 5.19 | 4.80 | 4.24 | 3.70 | 3.32 | 4.88 | 38.26        | 4.78        |
Table (4): Mean of chromosome long arm of the eight chromosomes for accessions alfalfa.

| Code | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | Total long arm | Mean long arm |
|------|------|------|------|------|------|------|------|------|----------------|--------------|
| 1    | 4.68 | 3.77 | 3.37 | 3.07 | 2.50 | 2.15 | 1.78 | 3.06 | 24.37          | 3.05         |
| 2    | 4.33 | 3.89 | 3.47 | 3.29 | 2.42 | 2.13 | 1.79 | 2.38 | 23.70          | 2.96         |
| 3    | 3.40 | 2.95 | 2.94 | 2.23 | 1.83 | 1.61 | 1.58 | 2.62 | 19.17          | 2.40         |
| 4    | 3.62 | 3.15 | 2.84 | 2.61 | 2.27 | 2.12 | 1.83 | 2.47 | 20.92          | 2.61         |
| 5    | 3.81 | 3.22 | 2.95 | 2.72 | 2.22 | 2.12 | 1.89 | 2.32 | 21.26          | 2.66         |
| 6    | 4.34 | 3.70 | 3.14 | 2.74 | 2.27 | 2.07 | 1.71 | 3.28 | 23.26          | 2.91         |
| 7    | 4.16 | 3.71 | 3.32 | 3.10 | 2.47 | 2.24 | 1.93 | 2.48 | 23.41          | 2.93         |
| 8    | 4.99 | 4.71 | 3.85 | 3.46 | 2.66 | 2.15 | 1.79 | 2.72 | 26.35          | 3.29         |
| 9    | 4.13 | 3.58 | 3.33 | 2.90 | 2.43 | 2.18 | 2.01 | 2.73 | 23.28          | 2.91         |
| 10   | 5.06 | 4.16 | 3.40 | 2.83 | 2.72 | 2.26 | 1.78 | 2.39 | 24.60          | 3.07         |
| 11   | 3.86 | 3.66 | 3.07 | 2.68 | 2.28 | 2.13 | 2.12 | 3.50 | 23.29          | 2.91         |
| 12   | 3.32 | 2.94 | 2.65 | 2.49 | 2.43 | 2.35 | 1.69 | 2.52 | 20.39          | 2.55         |
| 13   | 4.10 | 3.78 | 3.02 | 2.89 | 2.49 | 2.29 | 1.79 | 2.87 | 23.23          | 2.90         |
| 14   | 3.66 | 3.24 | 2.77 | 2.57 | 2.34 | 2.04 | 1.69 | 2.70 | 21.00          | 2.63         |
| 15   | 3.70 | 3.23 | 2.96 | 2.75 | 2.49 | 2.14 | 1.79 | 2.78 | 22.85          | 2.86         |
| 16   | 4.13 | 3.81 | 3.25 | 2.82 | 2.30 | 2.08 | 1.74 | 2.88 | 23.02          | 2.88         |
| 17   | 3.71 | 3.32 | 2.97 | 2.70 | 2.46 | 1.90 | 1.72 | 2.97 | 21.74          | 2.72         |
| 18   | 3.70 | 3.23 | 2.73 | 2.64 | 1.98 | 1.91 | 1.65 | 2.64 | 20.48          | 2.56         |
| 19   | 4.03 | 3.69 | 3.25 | 2.93 | 2.37 | 2.16 | 1.76 | 3.00 | 23.19          | 2.90         |
| 20   | 3.98 | 3.44 | 3.27 | 2.98 | 2.22 | 1.94 | 1.73 | 2.51 | 22.08          | 2.76         |
Table (5): Mean of chromosome short arm of the eight chromosomes for accessions alfalfa.

| Code | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | Total short arm | Mean short arm |
|------|------|------|------|------|------|------|------|------|-----------------|----------------|
| 1    | 3.45 | 2.51 | 2.16 | 2.04 | 2.19 | 1.98 | 1.60 | 2.82 | 18.75           | 2.34           |
| 2    | 2.97 | 2.65 | 2.37 | 1.93 | 2.33 | 1.88 | 1.63 | 2.25 | 18.02           | 2.25           |
| 3    | 2.15 | 1.77 | 1.63 | 1.22 | 1.60 | 1.52 | 1.48 | 2.44 | 13.81           | 1.73           |
| 4    | 2.20 | 1.91 | 1.92 | 1.87 | 2.06 | 1.98 | 1.68 | 2.22 | 15.84           | 1.98           |
| 5    | 2.40 | 2.08 | 2.06 | 1.81 | 2.00 | 1.90 | 1.77 | 2.15 | 16.18           | 2.02           |
| 6    | 2.97 | 2.54 | 2.22 | 1.93 | 2.03 | 1.90 | 1.54 | 2.98 | 18.11           | 2.26           |
| 7    | 2.93 | 2.37 | 2.29 | 2.17 | 2.27 | 1.97 | 1.75 | 2.39 | 18.14           | 2.27           |
| 8    | 3.54 | 3.04 | 2.83 | 2.18 | 2.48 | 1.98 | 1.53 | 2.58 | 20.17           | 2.52           |
| 9    | 3.03 | 2.44 | 2.27 | 1.98 | 2.17 | 1.99 | 1.81 | 2.60 | 18.30           | 2.29           |
| 10   | 3.49 | 2.77 | 2.22 | 1.96 | 2.55 | 1.56 | 1.64 | 2.31 | 18.50           | 2.31           |
| 11   | 2.94 | 2.59 | 2.21 | 1.93 | 2.29 | 1.95 | 1.90 | 3.22 | 19.03           | 2.38           |
| 12   | 2.44 | 2.19 | 1.87 | 1.79 | 1.62 | 1.51 | 1.60 | 2.33 | 15.36           | 1.92           |
| 13   | 2.99 | 2.48 | 2.19 | 2.01 | 2.13 | 2.01 | 1.73 | 2.64 | 18.18           | 2.27           |
| 14   | 2.43 | 2.30 | 1.91 | 1.81 | 1.79 | 1.77 | 1.59 | 2.38 | 15.98           | 1.99           |
| 15   | 2.70 | 2.40 | 2.02 | 2.01 | 2.00 | 1.90 | 1.75 | 2.49 | 16.42           | 2.05           |
| 16   | 2.31 | 2.11 | 1.74 | 1.82 | 2.02 | 1.87 | 1.67 | 2.79 | 16.34           | 2.04           |
| 17   | 2.08 | 1.70 | 1.58 | 1.46 | 1.39 | 1.79 | 1.55 | 2.89 | 14.43           | 1.80           |
| 18   | 2.00 | 1.84 | 1.73 | 1.39 | 1.79 | 1.57 | 1.54 | 2.63 | 14.49           | 1.81           |
| 19   | 2.54 | 2.18 | 2.08 | 1.93 | 2.11 | 1.96 | 1.64 | 2.94 | 17.38           | 2.17           |
| 20   | 2.57 | 2.13 | 1.92 | 1.82 | 2.01 | 1.75 | 1.56 | 2.38 | 16.74           | 2.09           |
Table (6): Mean of chromosome arm ratio, Centromeric index and Centromeric position of eight chromosomes for accessions alfalfa.

| Code | 1  | 2  | 3  |
|------|----|----|----|
|      | No. of Chromosomes | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position |
| 1    | 1.36 | 42.44 | S.M | 1.46 | 40.68 | S.M | 1.58 | 38.70 | S.M |
| 2    | 1.50 | 39.94 | S.M | 1.47 | 40.52 | S.M | 1.67 | 37.50 | S.M |
| 3    | 1.56 | 39.04 | S.M | 1.46 | 40.64 | S.M | 1.80 | 35.66 | S.M |
| 4    | 1.50 | 39.96 | S.M | 1.70 | 37.00 | S.M | 1.82 | 35.41 | S.M |
| 5    | 1.14 | 46.67 | M  | 1.04 | 49.04 | M  | 1.15 | 46.58 | M  |
| 6    | 1.08 | 48.02 | M  | 1.13 | 46.97 | M  | 1.06 | 48.55 | M  |
| 7    | 1.12 | 47.27 | M  | 1.10 | 47.66 | M  | 1.07 | 48.33 | M  |
| 8    | 1.00 | 50.00 | M  | 1.06 | 48.55 | M  | 1.08 | 48.18 | M  |

| Code | 4  | 5  | 6  |
|------|----|----|----|
|      | No. of Chromosomes | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position |
| 1    | 1.65 | 37.80 | S.M | 1.59 | 38.64 | S.M | 1.46 | 40.68 | S.M |
| 2    | 1.65 | 37.72 | S.M | 1.55 | 39.26 | S.M | 1.46 | 40.69 | S.M |
| 3    | 1.48 | 40.31 | S.M | 1.43 | 41.09 | S.M | 1.42 | 41.39 | S.M |
| 4    | 1.40 | 41.74 | S.M | 1.50 | 40.02 | S.M | 1.42 | 41.32 | S.M |
| 5    | 1.11 | 47.51 | M  | 1.11 | 47.34 | M  | 1.12 | 47.17 | M  |
| 6    | 1.07 | 48.22 | M  | 1.12 | 47.28 | M  | 1.09 | 47.86 | M  |
| 7    | 1.09 | 47.95 | M  | 1.07 | 48.36 | M  | 1.11 | 47.41 | M  |
| 8    | 1.11 | 47.35 | M  | 1.08 | 48.11 | M  | 1.10 | 47.55 | M  |

| Code | 7  | 8  | 9  |
|------|----|----|----|
|      | No. of Chromosomes | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position |
| 1    | 1.42 | 41.31 | S.M | 1.41 | 41.50 | S.M | 1.36 | 42.36 | S.M |
| 2    | 1.56 | 39.02 | S.M | 1.55 | 39.23 | S.M | 1.47 | 40.51 | S.M |
| 3    | 1.45 | 40.83 | S.M | 1.36 | 42.39 | S.M | 1.47 | 40.52 | S.M |
| 4    | 1.43 | 41.14 | S.M | 1.59 | 38.60 | S.M | 1.46 | 40.63 | S.M |
| 5    | 1.09 | 47.89 | M  | 1.07 | 48.22 | M  | 1.12 | 47.18 | M  |
| 6    | 1.14 | 46.73 | M  | 1.09 | 47.92 | M  | 1.09 | 47.76 | M  |
| 7    | 1.10 | 47.58 | M  | 1.17 | 46.10 | M  | 1.11 | 47.39 | M  |
| 8    | 1.04 | 49.10 | M  | 1.05 | 48.71 | M  | 1.05 | 48.85 | M  |
Table (6): Cont'

| No. of Chromosomes | Code | 10        |  | 11        |  | 12        |
|-------------------|------|-----------|---|-----------|---|-----------|
|                   |      | Arm ratio | Centromere | Centromeric index | Centromeric position | Arm ratio | Centromere | Centromeric index | Centromeric position | Arm ratio | Centromere | Centromeric index | Centromeric position |
| 1                 | 1    | 1.45      | 40.84 | S.M  | 1.31      | 43.26 | S.M  | 1.36      | 42.39 | S.M  |
| 2                 | 2    | 1.50      | 39.94 | S.M  | 1.41      | 41.44 | S.M  | 1.34      | 42.67 | S.M  |
| 3                 | 3    | 1.53      | 39.53 | S.M  | 1.39      | 41.88 | S.M  | 1.42      | 41.39 | S.M  |
| 4                 | 4    | 1.44      | 40.90 | S.M  | 1.39      | 41.82 | S.M  | 1.39      | 41.89 | S.M  |
| 5                 | 5    | 1.06      | 48.43 | M    | 0.99      | 50.14 | M    | 1.11      | 47.45 | M    |
| 6                 | 6    | 1.09      | 47.95 | M    | 1.12      | 47.24 | M    | 1.06      | 48.59 | M    |
| 7                 | 7    | 1.09      | 47.91 | M    | 1.09      | 47.92 | M    | 1.08      | 48.06 | M    |

| No. of Chromosomes | Code | 13        | 14 | 15        |
|-------------------|------|-----------|---|-----------|
|                   |      | Arm ratio | Centromere | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position |
| 1                 | 1    | 1.37      | 42.19 | S.M  | 1.51      | 39.91 | S.M  | 1.37      | 42.18 | S.M  |
| 2                 | 2    | 1.53      | 39.58 | S.M  | 1.41      | 41.55 | S.M  | 1.38      | 42.28 | S.M  |
| 3                 | 3    | 1.58      | 41.06 | S.M  | 1.42      | 43.00 | S.M  | 1.37      | 42.76 | S.M  |
| 4                 | 4    | 1.17      | 46.14 | M    | 1.07      | 48.24 | M    | 1.15      | 47.69 | M    |
| 5                 | 5    | 1.14      | 46.76 | M    | 1.15      | 46.42 | M    | 1.15      | 47.08 | M    |
| 6                 | 6    | 1.03      | 49.16 | M    | 1.09      | 48.68 | M    | 1.03      | 49.38 | M    |
| 7                 | 7    | 1.09      | 47.91 | M    | 1.13      | 46.88 | M    | 1.12      | 47.23 | M    |

| No. of Chromosomes | Code | 16        | 17 | 18        |
|-------------------|------|-----------|---|-----------|
|                   |      | Arm ratio | Centromere | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position |
| 1                 | 1    | 1.78      | 35.91 | S.M  | 1.78      | 35.93 | S.M  | 1.84      | 35.17 | S.M  |
| 2                 | 2    | 1.81      | 35.59 | S.M  | 1.96      | 33.77 | S.M  | 1.76      | 36.29 | S.M  |
| 3                 | 3    | 1.87      | 34.85 | S.M  | 1.88      | 34.78 | S.M  | 1.58      | 38.81 | S.M  |
| 4                 | 4    | 1.55      | 39.28 | S.M  | 1.85      | 35.07 | S.M  | 1.89      | 34.58 | S.M  |
| 5                 | 5    | 1.14      | 46.80 | M    | 1.16      | 46.18 | M    | 1.11      | 47.42 | M    |
| 6                 | 6    | 1.11      | 47.33 | M    | 1.06      | 48.52 | M    | 1.07      | 45.05 | M    |
| 7                 | 7    | 1.04      | 48.92 | M    | 1.11      | 47.31 | M    | 1.07      | 48.24 | M    |
| 8                 | 8    | 1.03      | 49.21 | M    | 1.03      | 49.31 | M    | 1.00      | 50.00 | M    |
Table (6): Cont’

| Code | 19 No. of Chromosomes | Arm ratio | Centromeric index | Centromeric position | Arm ratio | Centromeric index | Centromeric position |
|------|------------------------|-----------|-------------------|----------------------|-----------|-------------------|----------------------|
| 1    | 1.59                   | 38.66     | S.M               | 1.55                 | 39.23     | S.M               |
| 2    | 1.69                   | 37.15     | S.M               | 1.61                 | 38.25     | S.M               |
| 3    | 1.56                   | 39.02     | S.M               | 1.70                 | 37.07     | S.M               |
| 4    | 1.52                   | 39.73     | S.M               | 1.64                 | 37.89     | S.M               |
| 5    | 1.12                   | 47.10     | M                 | 1.10                 | 47.53     | M                 |
| 6    | 1.10                   | 47.58     | M                 | 1.11                 | 47.45     | M                 |
| 7    | 1.07                   | 48.35     | M                 | 1.09                 | 47.89     | M                 |
| 8    | 1.02                   | 49.44     | M                 | 1.06                 | 48.54     | M                 |
Fig. (1a): Mitotic metaphase and karyotype of chromosome of alfalfa at El-Kharga.

Fig. (1b): Mitotic metaphase and karyotype of chromosome of alfalfa at El-Dakhla.
Fig. (1c): Mitotic metaphase and karyotype of chromosome of alfalfa at El-Farafra.

Fig. (1d): Mitotic metaphase and karyotype of chromosome of alfalfa at Siwa.
