III.5 BIOCHEMICAL MARKERS ASSOCIATED WITH SOIL-BORN AND FOLIAR DISEASE RESISTANCE OF HIGH YIELDING CANOLA MUTANTS

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Abstract

Nineteen canola (*Brassica napus* L.) mutants developed by gamma ray in previous generations and their parental cultivars: Bactol, Linetta and Conny, were investigated for their resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases under greenhouse and field conditions during the winter seasons 2012-2013 and 2013-2014. Mutants CM1, CM2, CM8, CM12, CM14, CM17 and CM19 were the most resistant ones, while the three parental cultivars, CM9, CM16 and CM18 were the most susceptible ones. Based on protein profile of the 19 mutants and their parental cultivars by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique, SDS-PAGE profile showed some polypeptides associated with resistance and susceptibility to the diseases under investigation. Wide range of similarity index was observed among each parental cultivars and its developed mutants. The UPGMA based dendrogram showed that all the most resistant mutants to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases were gathered together as did the most susceptible ones.

Key Words: *Brassica napus* L., mutants, yield and yield components, charcoal rot, fusarium wilt, alternaria leaf spot, powdery mildew, SDS-PAGE, Biochemical markers and cluster analysis.

INTRODUCTION

Canola (*Brassica napus* L.) is an oilseed crop that has gained widespread acceptance worldwide due to the advantages of its healthy edible oil and high oil yield. Brassica species are now the second largest oilseed crops after soybean in the global oilseed-crop production, surpassing peanut, sunflower and cottonseed during the last two decades (Emrani, 2012). Oil containing only seven percent saturated fatty acids along with cholesterol lowering mono-unsaturated and poly-unsaturated fatty acids are the main components of canola seed oil (McDonald, 2011).

Canola was introduced to Egyptian agriculture during 1985 in order to produce its edible oil (El-Ahmer, 1989). The cultivated area is gradually increased and usually concentrated in the newly reclaimed lands, especially Nubaria, Toshka and East El-Owinat (Azzam and Abbas, 2005).

Useful genetic variability is the prerequisite for any breeding program. Besides conventional methods, induced mutation has been extensively used for developing

new genetic variation in crop plants. More than 2200 mutant cultivars of different crops with improved agronomic traits have been developed and released to the farmers for general cultivation all over the world (Maluszynski et al., 2000). Mutagenesis technique has also been successfully employed in rapeseed and mustard by the plant breeders to alter the genetic architecture of plant and isolate the possible mutants with desired economic plant characteristics such as plant height, number of pods per plant, number of seeds per pod, 1000-seed weight, seed yield, oil content and disease resistance (Azzam and Abbas, 2005; Azzam et al., 2008; and Azzam and Omran, 2012). Gamma irradiation has been used for improving disease resistance and increasing genetic variability in other oil crops such as peanut (Sorour et al., 1999; Azer et al., 2002; Azzam and El-Sawy, 2005; Khalifa et al., 2006; and Azzam et al., 2007b), sunflower (Azzam, 1993; Shabana et al., 1994; and Amer et al., 2001) and sesame (Azzam et al., 2007a).

The crop is usually subjected to infection by several soil-born and foliar diseases all over the world (Kolte, 1986), causing considerable losses in both seed and oil yield. In pioneer studies of El-Deeb et al. (1989) and Hilal et al. (1989) on rapeseed in Egypt, several fungal diseases were reported for the first time and induced charcoal rot (*Macrophomina phaseolina*) and fusarium wilt (*Fusarium oxysporium*). Moreover, losses in seed yield resulted from infection by charcoal rot and fusarium wilt ranged between 10.3 and 58.7% (Hilal et al., 1989 and Gouda, 1999).

Alternaria leaf blight (*Alternaria brassicae*), downy mildew (*Peronospora parasitica*), white rust (*Albugo candida*) and powdery mildew (*Erysiphe cruciferarum*) diseases, are most widely infected canola all over the world (Dang et al., 2000 and Sharma and Sharma, 2008). Among the foliar diseases, alternaria leaf spot and powdery mildew are considered serious diseases causing considerable losses on canola (*Brassica napus)* under changing agroclimatic conditions in Egypt (Draz, 1997 and Gouda, 1999)

Amounts of canola losses could be minimized by using resistant cultivars (Khalil, 2002) or through induced gamma-irradiation mutants (Azzam and Abbas, 2005; Azzam et al., 2008; and Azzam and Omran, 2012).

In the mid-1980s, the development of abundant molecular markers permitted the detection of marker associated with complex traits. Marker-assisted selection was then proposed as a means of exploiting markers to identify suitable genetic markers to trait of interest (e.g., productivity, disease resistance, abiotic stress tolerance, quality) that could be used in crop breeding through marker-assisted selection (MAS) to develop improved cultivars. Markers are usually based on DNA/RNA variation, but can also be biochemical or morphological. These applications will take advantage of cheaper costs of genotyping than of phenotyping. Thousands of marker-trait

associations have been reported for many traits in different plant species (Alt et al., 2005; Oliva et al., 2006; Khalifa et al., 2006; Azzam et al., 2007a & b; Abdel-Tawab et al., 2008; and Azzam et al., 2010).

The present study aimed to evaluate the behavior of 19 canola mutants and their parental cultivars under artificial and natural infection for charcoal rot and fusarium wilt pathogens as well as alternaria leaf spot and powdery mildew under natural infection and to determine specific biochemical markers associated with resistance and susceptibility to diseases under investigation.

MATERIALS AND METHODS

Nineteen high-yielding canola mutants tolerant to salinity and drought resulted from previous evaluation (Azzam et al., 2008 and Azzam and Omran, 2012) were evaluated in the present study, as shown in Table 1.

Table 1. The 19 canola mutants and their parental cultivar sources.

Genotype	Parent	Gamma ray dose
Bactol	kindly obtained from Oil Crops Res. Dep., Field Crops Res. Inst., ARC	
CM1	Bactol	400Gy
CM2	Bactol	400Gy
CM3	Bactol	400Gy
CM4	Bactol	400Gy
CM5	Bactol	600Gy
CM6	Bactol	600Gy
CM7	Bactol	600Gy
Linetta	German cultivar, kindly obtained from IPK Inst., Gatersleben, Germany	
CM8	Linetta	400Gy
CM9	Linetta	400Gy
CM10	Linetta	600Gy
CM11	Linetta	600Gy
CM12	Linetta	600Gy
Conny	German cultivar, kindly obtained from IPK Inst., Gatersleben, Germany	
CM13	Conny	400Gy
CM14	Conny	400Gy
CM15	Conny	400Gy
CM16	Conny	400Gy
CM17	Conny	600Gy
CM18	Conny	600Gy
CM19	Conny	600Gy

FIELD EVALUATION

Those 19 promising mutants along with their parental cultivars *i.e.* Bactol, Linetta and Conny were evaluated in field trial for their reaction against soil-born diseases *i.e.* charcoal rot and fusarium wilt and foliar diseases *i.e.* alternaria leaf spot and powdery mildew diseases in naturally heavily infested field conditions at Giza Research Station during two winter seasons (2012-2013 and 2013-2014) in RCBD design with three replicates; each plot consisted of five rows 3-m long and 20-cm apart. The field experiments were carried out in the first week of Nov. in both seasons. The recommended agricultural practices were applied in the experiments. At maturity, 10 plants were randomly selected from each plot to record yield and yield components:plant height (cm), number of branches plant^{-1}, number of siliques plant^{-1}, weight of siliques (g.), weight of seeds plant^{-1} and seed index (weight of 1000 seeds).

Disease assessment of charcoal-rot and wilt diseases was measured as percentages of diseased plants after 60, 90 days from planting and at harvest time according to specific disease symptoms (Charcoal-rot infection was expressed as root discoloration, black stem rot and pronounced reduction in root system of the infected plants. However, infected plants characterized by the internal vesicular discoloration wilt appearance and might be died and fell down was considered wilted). However, disease severity of alternaria leaf spot and powdery mildew was measured after 60 and 90 days from planting. Twenty five leaves from each plot were randomly chosen to determine disease severity of alternaria leaf spot and powdery mildew and was monitored using (0-5) scale (according to the method described by (Townsend and Heuberger, 1943 and Reuveni et al., 1997, respectively) and recorded as follows:
0 = no infection (leaves are completely healthy), 1= 1-5% area covered by the disease, 2 =6-10 % area covered, 3 =11-20 % area covered, 4= 21-30% area covered, 5= 31 - 100% area covered by the alternaria leaf spot. Disease severity index of alternaria leaf spot and powdery mildew was estimated using the following formula:

$$D.S.I = \frac{\Sigma (n \times v)}{ZN} \times 100$$

Where:

D.S.I= Disease severity index, n = Number of leaves in each category, v = Numerical value of each category, z = Numerical value of highest category and N = Total number of leaves in the sample.

GREENHOUSE EVALUATION

The 19 canola mutants and their parental cultivars were tested for their reaction against infection with either *Macrophomina phaseolina* or *Fusarium oxysporum,* the causal pathogens of charcoal rot and fusarium wilt diseases, respectively under artificial conditions in greenhouse at Giza Research Station in 2012-2013 season.

Fungal inoculation of *M. phaseolina* and *F. oxysporum* was prepared using sorghum-coarse sand-water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for two hr at 1.5 air pressure. The autoclaved media in glass bottles were inoculated separately using agar discs obtained from the periphery of 5-day old colony of each tested fungi and incubated at 26°C for two weeks and were then used for soil infection. Each fungal inoculum was added separately to the potted soil at the rate of 2% by weight, mixed thoroughly with the soil surface, then watered and left for one week before sowing. Seeds of each genotype were planted in the infected soil at the rate of ten seeds pot^{-1} (30 cm). Three pots were used for each particular treatment as replicates in RCBD design.

Disease assessment was measured as percentages of pre- and post-emergence damping-off after 15 and 45 days from sowing, respectively. Percentages of diseased plants infected with charcoal-rot or wilt diseases were estimated according to specific disease symptoms as mentioned before and recorded after 60 and 90 days from planting and at harvest time. Disease estimation in each stage was calculated based on number of seeds that were sown in each pot as follows:

$$\% \text{ Pre-emergence} = \frac{\text{Number of non germinated seeds}}{\text{Number of sown seeds}} \text{X } 100$$

$$\% \text{ Post-emergence} = \frac{\text{Number of dead seedlings}}{\text{Number of sown seeds}} \text{X } 100$$

$$\% \text{ Charcoal rot} = \frac{\text{Number of plants with charcoal rot symptoms}}{\text{Number of sown seeds}} \text{X } 100$$

$$\% \text{ Fusarium wilt} = \frac{\text{Number of plants with fusarim wilt symptoms}}{\text{Number of sown seeds}} \text{X } 100$$

$$\% \text{ Healthy plants} = \frac{\text{Number of healthy plants}}{\text{Number of sown seeds}} \text{X } 100$$

STATISTICAL ANALYSIS

The data of the two evaluated seasons were statistically analyzed as combined analysis for yield characteristics, while separately for disease evaluation by analysis of variance (ANOVA) using MSTAT-C program. The least significant difference (LSD) test at 0.05 was used to find out the significance of mean difference of various treatments (Gomez and Gomez, 1984).

SODIUM DODESYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

To find out biochemical marker associated with resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases through protein comparisons, seed storage protein (water soluble protein) of the 19 canola mutants along with their parental cultivars i.e. Bactol, Linetta and Conny were size fractionated separately (each cultivar with its mutants) based on the molecular weight using 10% SDS-PAGE according to Laemmli (1970). The gel was stained with Comassie blue R-250 solution overnight and distained using 40% methanol, 10% acetic acid and 50% distilled water until the bands were clearly visible. A 0.75 mm-thick vertical slab gel was cast and electrophoresed using the Bio Rad Mini-Protein II system. Gels were photographed and scored using gel documentation system manufactured by Alpha Ease FC (Alphimager 2200), U.S.A. The protein bands in each gel were analyzed by scoring the bands as present (1) or absent (0). A pairwise comparison of mutants and their parental cultivars was made and genetic similarities based on Jaccard's similarity coefficient were calculated among all possible pairs, using Simqual option and ordering in a similarity matrix. Based on the data, a dendrogram was prepared by the un-weighted pair group method with arithmetic mean (UPGMA) (Sokal and Sneath, 1963) by using a statistical software package "SPSS for MS Windows Release 15" that grouped the mutants and their parental cultivars into discrete clusters.

RESULTS AND DISCUSSION

YIELD AND YIELD COMPONENTS

Combined analysis of variance revealed significant differences among studied genotypes for plant height over the two combined seasons, while insignificant differences were observed between genotypes, seasons and their interaction for number of branches, as shown in Table 2.

Variation in plant heights was observed among each parent and its mutants. Plant height varied from 191.3 cm (CM4) to 125.8 (CM15). The plant height of Bactol cultivar and its mutants ranged from 191.3 and 151.7 cm (CM4 and CM6, respectively), while plant height of Linetta and its mutants ranged from 176.8 to 144.2cm (CM8 and CM12, respectively), meanwhile, it ranged from 180.0 to 125.8 cm of Conny and its mutants (CM18 and CM15, respectively). The mutant CM4 was the tallest genotype over the two winter seasons, while mutant CM15 (125.8cm) was observed as short stature than its parental cultivar (Conny) and over all studied genotypes and seasons, as shown in Table 2.

Table 2. Combined analysis of variance of plant height and number of branches for 19 canola mutants and their parental cultivars grown under field conditions in (2012/2013 and 2013/2014), as an average for the two seasons.

Genotype	Plant height	Number of branches
Bactol	155.0	8.0
CM1	172.7	6.3
CM2	166.3	7.0
CM3	170.7	7.5
CM4	191.3	6.3
CM5	163.3	7.2
CM6	151.7	6.2
CM7	179.8	6.2
Linetta	168.7	5.5
CM8	176.8	7.7
CM9	150.5	6.2
CM10	163.2	5.7
CM11	165.0	6.5
CM12	144.2	6.2
Conny	149.3	5.3
CM13	159.7	6.5
CM14	167.0	6.8
CM15	125.8	5.3
CM16	175.0	7.2
CM17	146.7	6.5
CM18	180.0	8.2
CM19	164.2	6.3
Mean	163.0	6.6
L.S.D. 0.05	Genotypes = 20.74 Seasons=N.S. Genotypes X Seasons = N.S.	Genotypes = *N.S. Seasons = N.S. Genotypes X Seasons = N.S.

N.S. = Not significant at 0.05 level of probability.

The dwarfness in plant height is associated with earliness in maturity (Olejniczak & Adamska, 1999), which is a desirable characteristic in crop plants. Das & Rahman (1988, 1994) and Shah et al. (1990) have isolated short statured mutants with high yield potential from mutagen treated populations of rapeseed and mustard. This confirmed that induced mutation through gamma rays and EMS has played a significant role in the alteration of plant architecture and selection of mutants with enhanced yield potential in rapeseed and mustard (Rahman, 1996 and Shah et al., 1999).

The combined mean values of number of siliques plant^{-1} and weight of siliques plant^{-1} (g) are illustrated in Table 3. Significant differences were found between the 19 canola mutants and their parental cultivars Bactol, Linetta and Conny over the two seasons for No. of siliques plant^{-1} and weight of siliques plant^{-1}.

These high variations reflect mutagens development. The highest number of siliques plant^{-1} was observed in mutants CM1 and CM4 over all studied genotypes, while the lowest number of siliques plant^{-1} was observed in two parental cultivars: Bactol and Conny that gave 283.8 and 284.3, respectively. As for weight of siliques per plant, Bactol and Conny recorded the lowest values giving 46.7 and 49.8 g., respectively. Mutants CM8, CM3 and CM6 were superior for weight of siliques per plant compared to their parental cultivars and all studied mutants giving 130.6, 113.8 and 100 g, respectively.

The increments in mean number of siliques per plant consequently increased seed yield plant^{-1}, which finally improved seed yield. In this respect, Chen et al. (1997) observed wide differences between rape lines with different genetic backgrounds and between different irradiation doses. Emrani et al. (2012) reported that the greater number of fruits per plant and seeds per fruit, as two important yield components produced at 1000 Gy-dose, led to higher seed weight. The high yielding mutants have been developed from gamma rays irradiated rapeseed and mustard (Shah et al., 1999 and Siddiqui et al., 2009).

The combined statistical analysis indicated significant differences among genotypes and the interaction between genotypes and growing seasons in seed yield and seed index, as shown in Table 4. All the mutants were significantly superior to their parental cultivars in weight of seeds per plant (seed yield g) and seed index (1000-seed weight). Highest seed yield per plant was observed in CM1 and CM8 (66.3 and 57.2 g, respectively) compared to all studied genotypes over the two growing seasons.

Table 3. Combined analysis of variance of No. of siliques plant^{-1} and weight of siliques plant^{-1} (g) for 19 canola mutants and their parental cultivars grown under field conditions in (2012/2013 and 2013/2014), as an average of the two seasons.

Genotype	No. of siliques plant^{-1}	Weight of siliques plant^{-1} (g)
Bactol	283.8	46.7
CM1	830.8	98.6
CM2	642.0	93.9
CM3	713.3	113.8
CM4	826.7	84.8
CM5	735.5	91.5
CM6	563.2	100.0
CM7	565.0	66.8
Linetta	379.5	69.5
CM8	719.3	130.6
CM9	616.7	75.4
CM10	399.7	68.5
CM11	617.5	68.2
CM12	791.7	82.14
Conny	284.3	49.8
CM13	481.5	89.4
CM14	671.2	91.3
CM15	409.8	59.7
CM16	574.2	71.7
CM17	610.7	79.6
CM18	475.5	79.2
CM19	703.5	86.9
Mean	586.2	81.7
L.S.D. 0.05	Genotypes = 234.21 Seasons= 73.85 Genotypes X Seasons = 346.41	Genotypes = 49.75 Seasons = N.S. Genotypes X Seasons = N.S

*N.S. = Not significant at 0.05 level of probability.

However, all parental cultivars recorded the lowest seed yield per plant giving 21.5, 20.6 and 17.9 g. for Conny, Bactol and Linetta, respectively. For seed Index, it gave 3.4, 3.0 and 2.9g for Linetta, Bactol and Conny, respectively. The highest seed index was observed in mutant CM13 (5.2g) followed by CM1 and CM2 (5.0g) compared with the remaining genotypes (Table 4). These mutants exhibited higher 1000-seed weight than their parental cultivars, which probably indicates an increase

in the size of grain as a result of induced mutation. These results are in conformity with the findings of Chauhan & Kumar (1986) and Shah and Rahman (1990) who have also reported the bold-seeded mutants in oilseed Brassica.

Table 4. Combined analysis of variance of weight of seeds plant^{-1} (g) and seed index (g) for 19 canola mutants and their parental cultivars grown under field conditions in (2012/2013 and 2013/2014), as an average of the two seasons

Genotypes	Weight of seeds plant^{-1} (g)	Seed index (g)
Bactol	20.6	3.0
CM1	66.3	5.0
CM2	41.0	5.0
CM3	33.8	4.3
CM4	33.0	3.8
CM5	38.3	3.9
CM6	37.1	4.6
CM7	37.3	4.5
Linetta	17.9	3.4
CM8	57.2	4.7
CM9	38.1	4.9
CM10	39.8	4.1
CM11	33.2	4.7
CM12	37.1	4.6
Conny	21.5	2.9
CM13	39.3	5.2
CM14	44.4	4.2
CM15	30.8	4.2
CM16	35.2	4.2
CM17	41.6	4.2
CM18	37.0	4.3
CM19	50.4	4.7
Mean	37.8	4.3
L.S.D. 0.05	Genotypes = 4.57 Seasons = N.S. Genotypes X Seasons = 7.10	Genotypes = 0.64 Seasons = *N.S. Genotypes X Seasons = 0.66

*N.S.= Not significant

Breeding for high yield is essentially based on the generation of new genotypes with improved yield and yield components or better agronomic traits, which are responsible for substantial increase in yield. Overall performance of the genotypes for yield and yield components indicate that the mutants CM1 and CM8, because of their high yield potential, held great promise to be a mutant cultivar. Moreover, this suggests that gamma rays irradiation with the dose range of 400 to 600 Gy can be fruitfully applied to develop new genotypes with high yield and other improved agronomic traits in canola (*B. napus*).

DISEASES EVALUATION

1-FIELD EVALUATION

The 19 canola mutants and their parental cultivars were evaluated against soil-born diseases *i.e.* charcoal rot and fusarium wilt and foliar diseases *i.e.* alternaria leaf spot and powdery mildew diseases in naturally heavily infected diseases under field conditions in 2012/2013 and 2013/2014 seasons.

1.1- REACTION TO CHARCOAL ROT AND FUSARIUM WILT DISEASES

Data presented in Table 5 show that the canola mutants and their parental cultivars reacted differently and significantly throughout the different diseases *i.e.* charcoal rot, and fusarium wilt. Generally, the data revealed that mutants *i.e.* CM1, CM2, CM4, CM5 and CM6, which were developed from Bactol cultivar and CM8, CM11 and CM12, which were developed from Linetta cultivar, as well as, CM14, CM17 and CM19 which were developed from Conny cultivar were the most resistant ones for charcoal rot and wilt diseases and gave the highest healthy plants with the range of 81.6-93.2% in 2012/2013 and 81.5-92.8% in 2013/2014. Regarding charcoal rot disease incidence, the most resistant ones were CM1, CM8, CM11, CM12, CM14, CM17 and CM19 in 2012/2013 with the range of 4.4-7.4% and CM8, CM14, CM17 and CM19 in 2013/2014 with the range of 4.0-6.2%, compared to their parental cultivars, which were the highest susceptible ones. However, mutants i.e. CM6, CM8, CM11, CM12, CM14, CM17 and CM19 were the most resistant ones in 2012/2013 (ranged from 2.0 to 6.3%) and CM1, CM2, CM5, CM8, CM12, CM14, CM17 and CM19 were the most resistant in 2013/2014 (ranged from 3.2 to 6.6%), for fusarium wilt disease incidence. On the other hand, the three parental cultivars i.e. Bactol, Linetta and Conny as well as, mutants CM9, CM15 and CM18 were the most susceptible ones, which gave the lowest healthy plants in the two successive winter seasons 2012/2013 and 2013/2014, while the other tested mutants were intermediate in their reaction.

Variable reaction to infection by the soil-born diseases studied appeared in values of disease incidence for the 19 canola mutants and their parental cultivars under field conditions. This reaction might be attributed to variation in genetic

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Variable reaction to infection by the soil-born diseases studied appeared in values of disease incidence for the 19 canola mutants and their parental cultivars under field conditions. This reaction might be attributed to variation in genetic

structure of the canola mutants tested, which in turn, affect root exudates and consequently change environmental circumstances at court of infection, which led to susceptibility or resistance reaction.

Table 5. Evaluation of 19 canola mutants and their parental cultivars against charcoal rot and fusarium wilt under field conditions in 2012/2013 and 2013/2014 seasons.

Genotype	Percentage of disease incidence					
	2012/2013			2013/2014		
	Charcoal roted plants	Fusarium wilted plants	Healthy plants	Charcoal roted plants	Fusarium wilted plants	Healthy plants
Bactol cultivar	23.3	16.3	60.4	31.2	25.8	43.0
CM1	6.9	8.3	84.8	10.3	3.3	86.4
CM2	12.3	7.7	80.0	9.3	3.8	86.9
CM3	17.9	13.8	68.3	15.6	7.9	76.5
CM4	12.5	11.1	76.4	8.3	8.5	83.2
CM5	13.2	9.4	77.4	8.7	6.1	85.2
CM6	12.1	6.3	81.6	8.7	7.4	83.9
CM7	11.1	10.3	78.6	7.2	11.3	81.5
Linetta cultivar	23.0	18.6	58.4	24.1	15.8	60.1
CM8	5.0	3.8	91.2	6.2	4.3	89.5
CM9	15.4	19.3	65.3	18.6	19.6	61.8
CM10	12.0	10.5	77.5	17.7	11.5	70.8
CM11	7.4	3.2	89.4	9.2	8.7	82.1
CM12	4.4	4.8	90.8	8.8	6.6	84.6
Conny cultivar	25.6	17.5	56.9	28.7	15.8	55.5
CM13	13.6	13.3	73.1	9.0	7.3	83.7
CM14	4.7	5.8	89.5	5.0	3.9	91.1
CM15	23.8	17.8	58.4	19.8	18.3	61.9
CM16	13.8	8.8	77.4	19.4	12.9	67.7
CM17	6.6	4.2	89.2	4.3	6.3	89.4
CM18	20.9	16.0	63.1	23.3	17.5	59.2
CM19	4.8	2.0	93.2	4.0	3.2	92.8
L.S.D. 0.05	6.23	4.94	12.43	6.61	5.03	13.19

1.2- REACTION TO ALTERNARIA LEAF SPOT AND POWDERY MILDEW DISEASES

The tested 19 canola mutants and their parental cultivars reacted differently and significantly throughout alternaria leaf spot and powdery mildew diseases (Table 6).

Table 6. Evaluation of nineteen canola mutants and their parental cultivars against alternaria leaf spot and powdery mildew under field conditions during two winter seasons.

Genotype	Percentage of disease severity			
	2012-2013		2013-2014	
	Alternaria leaf spot	Powdery mildew	Alternaria leaf spot	Powdery mildew
Bactol cultivar	29.9	32.8	31.3	35.7
CM1	6.9	11.6	9.1	14.3
CM2	12.9	16.7	14.8	18.9
CM3	23.4	28.7	22.7	31.9
CM4	12.1	17.5	14.7	20.8
CM5	20.6	19.4	25.2	22.1
CM6	26.3	27.6	29.6	29.4
CM7	17.1	35.3	19.9	37.8
Linetta cultivar	20.3	37.5	23.2	41.4
CM8	4.8	9.5	5.7	11.5
CM9	26.7	31.3	33.8	38.1
CM10	18.0	23.3	16.8	26.2
CM11	14.7	26.4	17.1	27.9
CM12	2.4	10.7	3.2	12.6
Conny cultivar	28.5	36.6	30.4	42.8
CM13	23.5	15.1	26.9	19.2
CM14	11.8	13.5	13.2	16.8
CM15	23.1	28.9	25.3	31.8
CM16	26.3	33.2	23.4	35.7
CM17	19.7	16.7	16.6	14.6
CM18	18.3	29.0	17.3	33.9
CM19	1.6	5.7	1.3	4.3
L.S.D. 0.05	8.22	10.39	8.88	11.61

For alternaria leaf spot disease severity, mutants *i.e.* CM1, CM2, CM4, CM8, CM12, CM14 and CM19 were the most resistant ones in the two seasons 2012/2013 (1.6-12.9%) and 2013/2014 (1.3-14.8%), with no significant differences between them. However, similar trend was observed in powdery mildew disease where, CM1, CM2, CM8, CM12, CM13, CM14, CM17 and CM19 were the most resistant ones in 2012/2013 (5.7–16.7%) and (4.3-16.8%) in 2013/2014, respectively with no significant differences between them. The obtained results concluded that mutants *i.e.* CM1, CM2, CM4, CM8, CM12, CM4, CM17 and CM19 were the most resistant ones for alternaria leaf spot and powdery mildew diseases in both seasons. The present results are in agreement with those found in Egypt by Draz (1997), Gouda, (1999) and Khalil, (2002). Dang et al. (2000) evaluated 36 Cruciferae (Brassicaceae) genotypes belonging to different brassicas for resistance to Alternaria leaf blight (*Alternaria brassicae*), downy mildew (*Peronospora parasitica*), white rust (*Albugo candida*) and

powdery mildew (*Erysiphe cruciferarum*) diseases during three seasons (1994-96) and reported that seven cultivars/genotypes (*B. alba* [*Sinapis alba*], *B. carinata* (HC-1), *B. juncea* (DIR-1507 and DIR-1522) and *B. napus* (GS-7027, Midas and Tower) had stable and multiple disease resistance. In addition, 13 genotypes belonging to different species possessed a fair degree of stable multiple disease resistance but to a lesser extent.

2-GREENHOUSE EVALUATION

In these experiments, 19 canola mutants and their parental cultivars were evaluated against disease development under greenhouse conditions in 2012/2013 season in soil infested with either *M. phaseolina*, or *F. oxysporum* the causal pathogens of charcoal rot and fusarium wilt diseases, respectively.

2.1- REACTION TO *M. phaseolina*

Data in Table 7 illustrated that the tested canola mutants and their parental cultivars reacted differently and significantly throughout the different stages of disease development (Pre-, post-emergence damping off, charcoal rot and healthy plants). Percentages of pre-, post-emergence damping off, charcoal rotted and healthy plants ranged from 0.0 to 23.3%, 0.0 to 23.3%, 3.3 to 30.0%, and 30.0 to 93.3%, respectively. The data also indicated that mutants CM1, CM2, CM6, CM8, CM10 and CM12 were the most resistant ones, which gave the lowest pre-emergence damping off (with range of 0.0-3.3%). Mutants CM1, CM8, CM13, CM17 and CM19 were the most resistant ones against post-emergence damping off (0.0-3.3%). On the other hand, mutants CM2, CM8, CM12, CM14, CM17 and CM19 were the most resistant ones for charcoal rotted (3.3-6.7%), however, mutants *i.e.* CM1, CM8, CM12, CM17 and CM19 gave the highest healthy plants (83.3-93.3%). The parental cultivars: Bactol, Linetta and Conny, as well as mutants *i.e.* CM3, CM16 and CM18 were the most susceptible ones, which gave the highest pre-, post-emergence damping off, charcoal rotted plants and the lowest healthy plants (ranged 30.0-43.3% healthy plants). The other tested mutants were intermediate in resistance.

2.2- Reaction to *F. oxysporum*

The evaluated canola mutants and their parental cultivars were reacted differently and significantly throughout the different stages of disease development (Pre-, post-emergence damping off, fusarium wilted and healthy plants). Percentages of disease incidence at different stages ranged from 0.0-23.3%, 0.0-20.0%, 0.0-30.0% and 33.3-96.7% for pre-, post-emergence damping off, as well as wilted and healthy plants, respectively (Table 8).

The results in Table 8 indicated that mutants CM1, CM2, CM6, CM11, CM13 and CM17 were the most resistant ones for pre-emergence damping off. However,

mutants CM1, CM2, CM6, CM8, CM12, CM13, CM14, CM17 and CM19 were the best resistant ones against post-emergence damping off. Meanwhile, mutants CM1, CM8, CM12, CM14, CM17 and CM19 were the most resistant ones for wilted plants (ranged from 0.0-3.3% for pre-, post-emergence damping off and fusarium wilt, respectively). Whereas, mutants CM1, CM2, CM6, CM8, CM12, CM17 and CM19 gave the highest resistant mutants giving the highest healthy plants (ranged 86.7-96.7% with no significant differences between them). On the other hand, the parental cultivars Bactol and Conny were the most susceptible ones, which gave the highest pre, post-emergence damping off and wilted plants and produced the lowest healthy plants (33.3%). The other tested genotypes were intermediate in this respect.

Table 7. Evaluation of 19 canola mutants and their parental cultivars against *M. phaseolina* under greenhouse conditions in 2012/2013 season.

Genotype	Disease incidence %			Healthy plants %
	Damping off		Charcoal rooted plants	
	Pre- emergence	Post-emergence		
Bactol cultivar	23.3	20.0	26.7	30.0
CM1	0.0	3.3	10.0	86.7
CM2	3.3	10.0	6.7	80.0
CM3	13.3	20.0	30.0	36.7
CM4	16.7	23.3	13.3	46.7
CM5	13.3	16.7	13.3	56.7
CM6	0.0	16.7	13.3	70.0
CM7	6.7	20.0	20.0	53.3
Linetta cultivar	13.3	30.0	23.3	33.3
CM8	0.0	0.0	6.7	93.3
CM9	20.0	13.3	16.7	50.0
CM10	3.3	13.3	13.3	70.0
CM11	13.3	16.7	10.0	60.0
CM12	3.3	6.7	6.7	83.3
Conny cultivar	20.0	23.3	26.7	30.0
CM13	13.3	3.3	10.0	73.3
CM14	6.7	13.3	3.3	76.7
CM15	13.3	16.7	16.7	53.3
CM16	23.3	20.0	26.7	30.0
CM17	6.7	0.0	6.7	86.7
CM18	16.7	10.0	30.0	43.3
CM19	6.7	0.0	3.3	90.0
L.S.D. 0.05	5.48	6.51	6.28	12.04

Table 8. Evaluation of 19 canola mutants and their parental cultivars against *F. oxysporum* under greenhouse conditions in 2012/2013 season.

Mutant No.	Disease incidence %			Healthy plant %
	Damping off		Wilted plant	
	Pre- emergence	Post- emergence		
Bactol cultivar	16.7	20.0	30.0	33.3
CM1	3.3	0.0	0.0	96.7
CM2	3.3	3.3	6.7	86.7
CM3	13.3	6.7	23.3	56.7
CM4	6.7	10.0	10.0	73.3
CM5	13.3	13.3	6.7	66.7
CM6	3.3	0.0	10.0	86.7
CM7	6.7	10.0	6.7	76.7
Linetta cultivar	20.0	13.3	10.0	56.7
CM8	6.7	0.0	0.0	93.3
CM9	10.0	6.7	16.7	66.7
CM10	10.0	3.3	6.7	80.0
CM11	0.0	6.7	10.0	83.3
CM12	6.7	0.0	3.3	90.0
Conny cultivar	23.3	16.7	26.7	33.3
CM13	3.3	3.3	10.0	83.3
CM14	10.0	3.3	3.3	83.3
CM15	6.7	10.0	6.7	76.7
CM16	16.7	13.3	10.0	60.0
CM17	3.3	0.0	3.3	93.3
CM18	13.3	16.7	6.7	63.3
CM19	6.7	0.0	3.3	90.0
L.S.D. 0.05	4.58	3.95	5.10	12.28

Charcoal rot and fusarium wilt diseases were recorded on the growing canola (rapeseed) plants in Egypt, causing considerable losses in yield components (Hilal et al., 1989; El-Deeb et al., 1989; Gouda, 1999 and Khalil, 2002). The present results are in harmony with those found in Egypt by Draz (1997), Gouda, (1999) and Khalil, (2002). They revealed that there is clear variation in susceptibility of rapeseed genotypes to the fungal diseases studied including charcoal rot and fusarium wilt.

Biochemical markers associated with disease resistance

The electrophoretic banding patterns of proteins extracted from the seeds of the 19 promising mutants along with their parental cultivars Bactol, Linetta and Conny are shown in Figs. 1, 2 and 3 and their densitometric analysis are illustrated in Tables 9, 10 and 11, where the presence and absence of bands were assessed with (1) and (0), respectively.

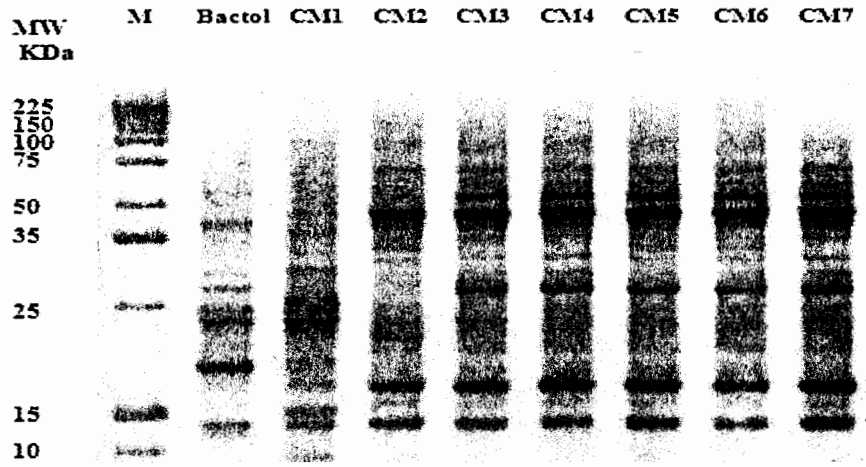

Fig. 1. SDS-PAGE 10% of Bactol cultivar (parent) and its developed mutants.
M= Protein marker.

SDS-PAGE of total soluble proteins showed 26 polypeptide bands with varying intensity and heterogenous among Bactol cultivar and its mutants, with molecular weights (MW) ranging from about 8.65 to 105.69 KDa, which were not necessarily present in all genotypes. Data showed one common band (monomorphic) in Bactol cultivar and all its mutants at molecular weight of 10.95 KDa and three monomorphic bands appeared only in the mutants developed from Bactol cultivar with molecular weight of 61.01, 15.28 and 12.48 KDa, while the remaining bands were polymorphic with 93.3% polymorphism (Table 9 and Fig. 1). The high magnitude of variability given in the mutants increased the genetic variance and consequently further practicing selection within these mutants is possible.

Table 9. Densitometric analysis for SDS seed storage protein (water soluble fraction) of Bactol cultivar (parent) and its developed mutants.

Band No	MW KDa	Bactol	CM1	CM2	CM3	CM4	CM5	CM6	CM7
1	105.69	0	1	1	0	0	0	0	0
2	102.35	0	1	1	0	0	0	0	0
3	70.76	0	0	1	1	1	1	1	1
4	61.01	0	1	1	1	1	1	1	1
5	56.41	1	0	0	0	0	0	0	0
6	54.47	0	0	1	1	1	1	1	1
7	53.07	1	0	0	0	0	0	0	0
8	52.15	0	1	0	0	0	0	0	0
9	49.50	1	0	0	1	1	0	0	0
10	45.75	0	0	0	0	0	1	1	0
11	44.19	0	1	1	0	0	0	0	0
12	41.57	0	0	1	1	1	1	1	1
13	38.23	0	1	0	1	1	1	1	1
14	37.44	1	0	0	0	0	0	0	0
15	35.53	0	0	0	1	0	1	1	0
16	33.14	1	0	0	1	1	1	1	1
17	30.37	0	1	0	0	0	0	0	0
18	28.28	1	1	1	0	0	1	1	1
19	26.18	0	1	0	1	0	0	0	0
20	24.00	1	0	0	1	1	1	1	1
21	23.58	0	1	1	0	0	0	0	0
22	17.68	1	0	0	0	0	0	0	0
23	15.28	0	1	1	1	1	1	1	1
24	12.48	0	1	1	1	1	1	1	1
25	10.95	1	1	1	1	1	1	1	1
26	8.65	0	1	1	0	0	0	0	0
Total No of Bands		9	14	13	13	11	13	13	11

Five unique bands were detected and scored in the most resistant mutants CM1 and CM2 at the molecular weight of 105.69, 102.35, 44.19, 23.58 and 8.65 KDa. These bands could be used as positive biochemical markers for resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases.

For Linetta cultivar and its mutants (Table 10 and Fig. 2), the results of SDS-PAGE of seed storage protein revealed a total number of 16 bands with molecular weights (MW) ranging from 14.20 to 69.50 KDa, which were not necessarily present in all genotypes. Data showed two common bands (monomorphic) at the molecular weight of 17.34 and 14.20 KDa, while the remaining bands were polymorphic with

87.5% polymorphism. There is no resemblance between Linetta cultivar and its mutants and each was characterized by a unique fingerprint.

Results of disease evaluation proved that mutants CM8 and CM12, which were developed from Linetta cultivar were the most resistant ones among the five canola mutants and their parental cultivar Linetta for the most tested diseases. On the other hand, Linetta and CM9 were the most susceptible ones in this respect. Two unique bands were detected and scored in the most resistant mutant CM8 and CM12 at the molecular weight of 20.94 and 18.26 KDa. These bands could be used as positive biochemical markers for resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases. On the other hand, four polypeptides with molecular weight of 69.50, 59.31, 50.00 and 15.14 KDa were absent in CM8 and CM12 (the most resistant mutants). These bands could be used as negative biochemical markers for resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases.

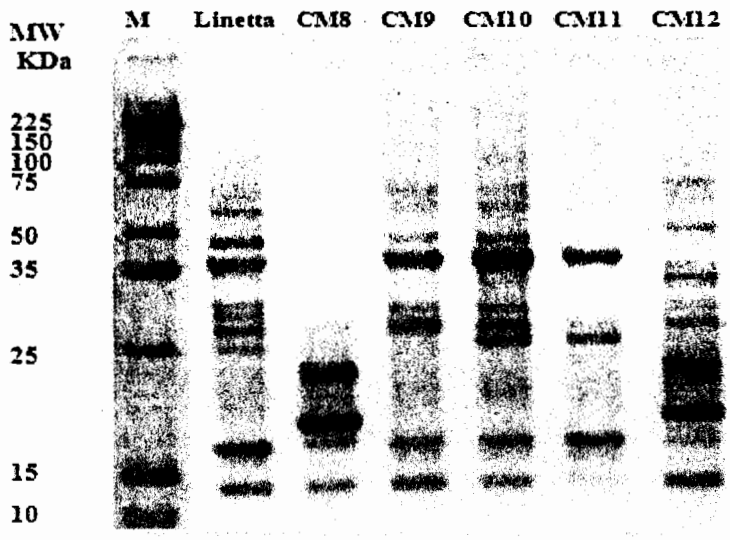

Fig.2. SDS-PAGE 10% for seed storage protein (water soluble protein) of Linetta cultivar (parent) and its developed mutants. M= Protein marker.

Data concerning SDS–protein profile of Conny cultivar and its developed mutants is given in Fig. (3). Thirty-one polymorphic bands were recorded in seed protein patterns with 100% polymorphism (Table 11). This high polymorphism, which reflects high variation among Conny cultivar and its developed mutants is in contrary with results obtained by Ahmed and Afiah (2008), who have detected a slight variation in protein banding pattern of nine lines of two ancestors of canola investigated under three environmental conditions. The low level of protein

polymorphism could be attributed to the conservative nature of the seed protein. This conclusion is in accordance with Nisar et al. (2007) and Sultana and Ghafoor (2008).

Table 10. Densitometric analysis for SDS seed storage protein (water soluble fraction) of Linetta cultivar (parent) and its developed mutants.

Band No	MW KDa	Linetta	CM8	CM9	CM10	CM11	CM12
1	69.50	1	0	1	1	1	0
2	59.31	1	0	1	1	1	0
3	50.00	0	0	0	0	0	1
4	41.61	1	0	1	1	1	0
5	35.22	0	0	1	1	1	0
6	34.12	0	0	0	0	0	1
7	32.50	0	0	0	0	0	1
8	28.84	1	0	1	1	0	0
9	27.33	1	0	1	1	1	1
10	26.11	1	0	0	1	1	0
11	25.20	1	0	0	0	0	0
12	20.94	0	1	0	0	0	1
13	18.26	0	1	0	0	0	1
14	17.34	1	1	1	1	1	1
15	15.14	1	0	1	1	1	0
16	14.20	1	1	1	1	1	1
Total No of Bands		10	4	9	10	9	8

The polymorphic bands were detected at approximately molecular mass ranging between 75.01 and 12.00 KDa. Two unique bands were scored in the most resistant mutants: CM14, CM17 and CM19 at molecular masses of 40.98 and 17.23 KDa. These bands could be used as positive biochemical markers for resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases. On the other hand, one polypeptide of molecular weight of 39.50 KDa was absent in CM16 and CM18 (the most susceptible mutants). These bands could be used as positive biochemical markers for susceptibility to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases. Occasionally, variation was also observed in density or sharpness of a few bands, but this variation was not taken into consideration.

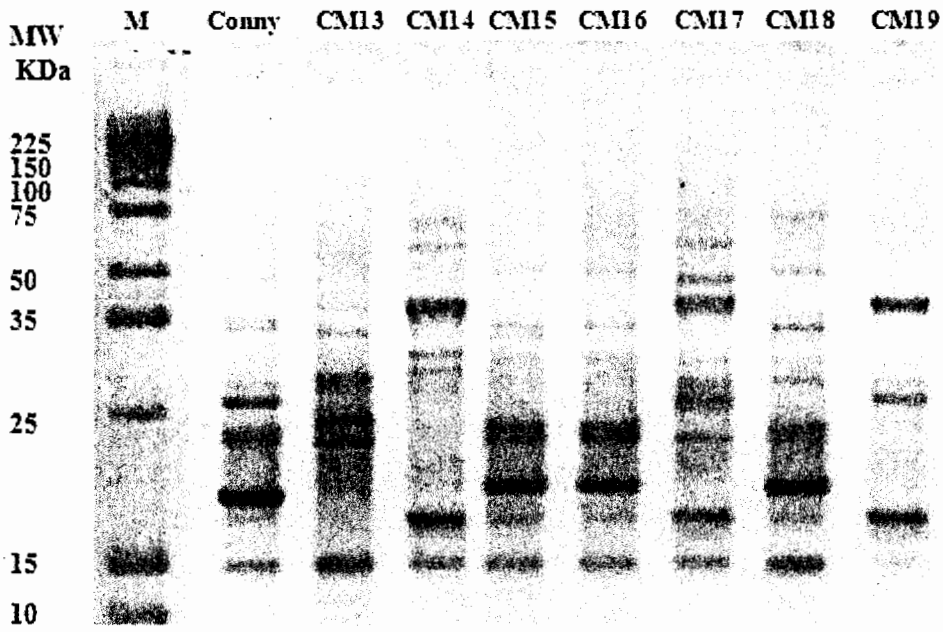

Fig. 3. SDS-PAGE 10%of Conny cultivar (parent) and its developed mutants.
M= Protein marker

Jaccard's similarity coefficient values were found to be in the range of 0.174 and 0.975 (Table 12), indicating a wide genetic base among Bactol cultivar and its developed mutants. Jaccard's similarity coefficient values between the parental cultivar Bactol and both of CM1 and CM2 that considered as the most resistant mutants to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases recorded 0.174 and 0.182, respectively. On the other hand, the high similarity index indicating the limitation of variation among the irradiated genotypes and corresponding parent and vice versa. Concerning the Jaccard's similarity coefficient values among all mutants developed through irradiation of Linetta cultivar and the parental cultivar Linetta ranged from 0.286 to 0.947, respectively as shown in Table 13. The similarity coefficients value between the parental cultivar Linetta and both of CM8 and CM12 that considered as the most resistant mutants to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases recorded 0.286 and 0.333, respectively, as shown in Table 13.

Regarding the Jaccard's similarity coefficient values among all mutants developed through irradiation of Conny cultivar and Conny cultivar itself ranged from 0.100 to 0.875, as shown in Table 14.

Table 11. Densitometric analysis for SDS seed storage protein (water soluble fraction) of Conny cultivar (parent) and its developed mutants.

Band No	MW KDa	Conny	CM13	CM14	CM15	CM16	CM17	CM18	CM19
1	75.01	0	1	0	0	0	0	0	0
2	74.30	0	0	0	0	0	0	0	1
3	73.42	0	1	1	0	0	0	0	0
4	70.50	0	0	0	0	0	1	0	0
5	69.11	0	0	1	0	0	0	0	0
6	65.20	0	1	0	0	0	0	0	0
7	51.22	0	0	1	1	1	0	0	1
8	50.43	1	0	0	0	0	0	0	0
9	49.29	0	0	0	0	0	1	0	0
10	48.50	0	1	1	0	0	0	0	0
11	40.98	0	0	1	0	0	1	0	1
12	39.50	0	0	0	0	1	0	1	0
13	35.33	1	0	0	1	1	0	1	0
14	33.59	1	1	0	1	0	0	0	0
15	31.10	1	0	0	0	0	0	0	0
16	30.19	0	0	1	1	1	1	1	0
17	29.36	0	0	1	1	0	0	0	0
18	28.89	1	1	0	0	1	0	1	1
19	27.19	0	0	0	0	0	1	0	1
20	26.31	1	0	0	0	0	0	0	0
21	25.45	0	1	0	0	0	0	0	0
22	24.36	1	0	0	1	1	0	1	0
23	23.53	0	0	0	0	0	1	0	0
24	22.39	1	1	0	1	1	0	0	0
25	21.10	0	0	0	1	1	0	1	0
26	19.18	1	0	0	0	0	0	0	0
27	18.51	1	0	0	0	0	0	0	0
28	17.23	0	0	1	0	0	1	0	1
29	16.57	0	0	0	0	0	0	0	1
30	15.49	1	1	1	1	1	1	1	0
31	12.00	0	1	0	0	0	0	0	0
Total No of Bands		11	10	9	9	9	8	7	7

Table 12. Similarity matrix for Jaccard's coefficient based on SDS-PAGE banding pattern for Bactol cultivar and its developed mutants.

Genotype	Bactol	CM1	CM2	CM3	CM4	CM5	CM6
CM1	0.174						
CM2	0.182	0.741					
CM3	0.314	0.444	0.538				
CM4	0.400	0.450	0.583	0.917			
CM5	0.364	0.444	0.625	0.846	0.823		
CM6	0.364	0.434	0.615	0.836	0.813	0.975	
CM7	0.400	0.480	0.667	0.833	0.909	0.927	0.917

Table 13. Similarity matrix for Jaccard's coefficient based on SDS-PAGE banding pattern for Linetta cultivar and its developed mutants.

Genotype	Linetta	CM8	CM9	CM10	CM11
CM8	0.286				
CM9	0.842	0.308			
CM10	0.900	0.286	0.947		
CM11	0.842	0.308	0.889	0.947	
CM12	0.333	0.667	0.353	0.333	0.353

Table 14. Similarity matrix for Jaccard's coefficient based on SDS-PAGE banding pattern for Conny cultivar and its developed mutants.

Genotypes	Conny	CM13	CM14	CM15	CM16	CM17	CM18
CM13	0.381						
CM14	0.100	0.316					
CM15	0.500	0.316	0.444				
CM16	0.500	0.316	0.333	0.778			
CM17	0.105	0.111	0.421	0.235	0.235		
CM18	0.444	0.235	0.250	0.625	0.875	0.267	
CM19	0.118	0.118	0.375	0.125	0.250	0.400	0.143

The dendrogram of Bactol, Linetta and Conny cultivars and their developed mutants, are presented in Figs 4, 5 and 6, respectively showing the genetic relationships between each parental cultivar and its developed mutants. Concerning Bactol, it was separated alone into main clusters, while all developed mutants were separated in another main cluster, as shown in Fig. (4). CM1 and CM2, the most resistant mutants to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases, were separated alone in one of the two sub-subclusters.

Regarding Linetta, all genotypes were separated into two main clusters, one of them included the most resistant mutants to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases, CM8 and CM12, while the other mutants and their parental cultivar Linetta were separated in the other main cluster. The first main cluster was separated into two sub-subclusters; one of them included Linetta cultivar alone, while the othe sub-subcluster separated the most susceptible mutant CM9 alone and the moderate resistant mutants together (CM10 and CM11), as shown in Fig. (5). Associations among the Conny cultivar and its developed mutants revealed by UPGMA cluster analysis based on SDS-PAGE are presented in Fig. (6) and it grouped the genotypes into two clusters. Cluster no. one included CM14, CM17 and CM19 (the most resistant mutants to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases), whereas the remaining mutants and their parental variety were separated consequently to group the more susceptible mutants CM16 and CM18 together at the end.

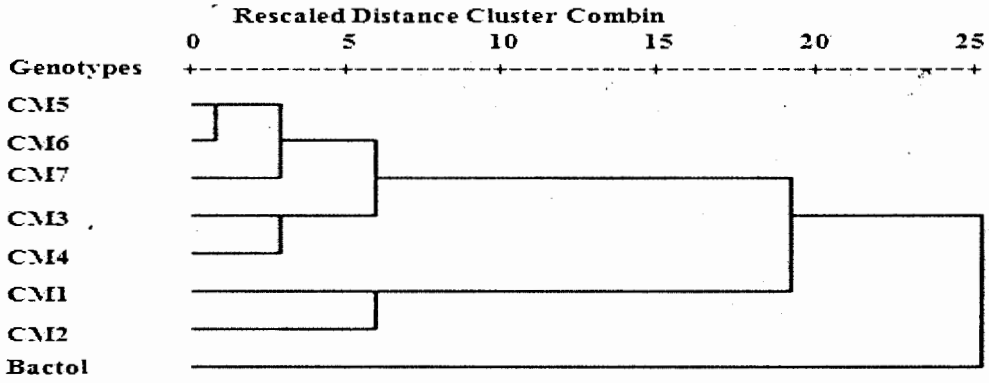

Fig.4. A dendrogram showing the genetic distance among Bactol cultivar and its developed mutants based on Jaccard's similarity coefficient of SDS-PAGE banding pattern.

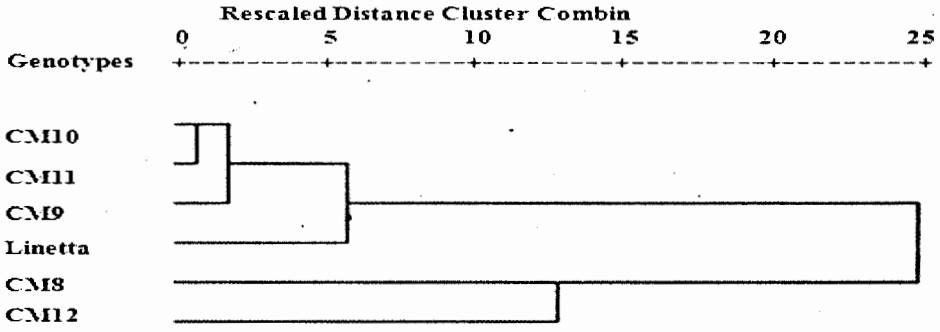

Fig.5. A dendrogram showing the genetic distance among Linetta cultivar and its developed mutants based on Jaccard's similarity coefficient of SDS-PAGE banding pattern

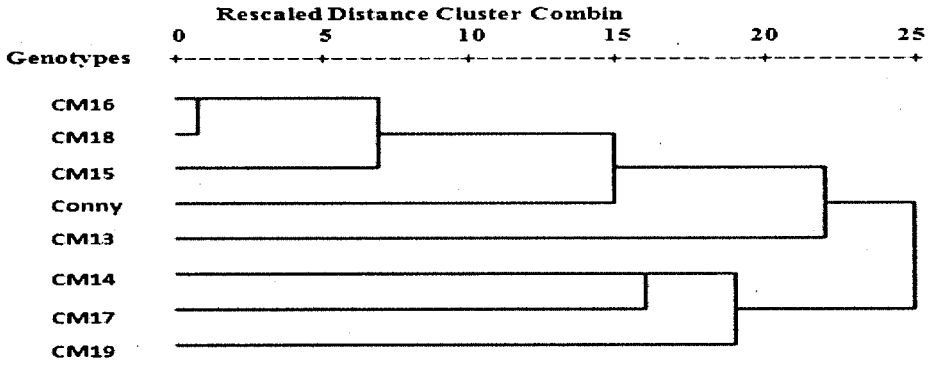

Fig.6. A dendrogram showing the genetic distance among Conny cultivar and its developed mutants based on Jaccard's similarity coefficient of SDS-PAGE banding pattern

It could be concluded that polypeptides with molecular weight of 105.69, 102.35, 44.19, 40.98, 23.58, 20.94, 18.26, 17.23 and 8.65 KDa could be used as positive biochemical markers for resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases, while polypeptides with molecular weight of 69.50, 59.31, 50.00 and 15.14 KDa could be used as negative biochemical markers for resistance to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases. On the other hand, polypeptides of molecular weight of 39.50 and 28.84 KDa could be used as positive biochemical markers for susceptibility to charcoal rot, fusarium wilt, alternaria leaf spot and powdery mildew diseases in canola genotypes. These result confirm results with other field crops used SDS-PAGE to develop biochemical markers associated with economic traits such as those of El-Menshawi et al. (2003) who developed biochemical markers associated with salt tolerance in sorghum, as well as, Khalifa et al. (2006) who found biochemical markers associated with disease resistance to damping-off and root-rot diseases of peanut mutants. Also, Azzam et al. (2007a) found biochemical genetic markers for levels of resistance to Cowpea Aphid Borne Mosaic Potyvirus in sesame mutants with molecular weight 82.0 and 38.0 KDa, as well as, Abd El-Naby et al. (2014) who developed biochemical markers associated with levels of resistance to damping-off diseases in alfalfa.

For better understanding of the presence of genetic variability in canola mutants and generations and consequently more efficient utilization of existing variability for improvement of the crop in Egypt, more biochemical and molecular data is required. Conclusively, this study is used to identify biochemical markers that could be used in crop breeding through marker-assisted selection (MAS) and confirmed that biochemical markers provided useful information for understanding the intra- and inter-specific variations and genetic relationships of canola genotypes for selecting the best for disease resistance and yield improvement.

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٣-٥ المعلمات البيوكيميائية المرتبطة بمقاومة أمراض التربة والمجموع الخضري لطفرات الكانولا ذات الإنتاجية العالية

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تم تقييم تسعة عشر طفرة كانولا ,والتي تم استنباطها من التشعيع بأشعة جاما فى أجيال سابقة وآبائهم لمقاومتهم لبعض أمراض التربة التي تصيب المجموع الجذري مثل مرضي العفن الفحمي والذبول الفيوزاريومي تحت ظروف الصوبة والحقل في موسمي ٢٠١٢/٢٠١٣ و ٢٠١٣/٢٠١٤ وبعض الأمراض التي تصيب المجموع الخضري مثل مرضَي تبقع الأوراق والبياض الدقيقي تحت ظروف الحقل في موسمي ٢٠١٢/٢٠١٣ و ٢٠١٣/٢٠١٤. وقد تفاوتت الطفرات المختلفة في مقاومتها للإصابة بالأمراض المختبرة. وكانت الطفرات CM12, CM8, CM2, CM1، CM14، CM17 , CM19 هي الأكثر مقاومة بينما كانت الطفرات CM9 ، CM16 و CM18 والآباء الثلاثة هي التراكيب الوراثية الأكثر قابلية للإصابة بمعظم الأمراض المختبرة في هذا الصدد. وقد وجد أن تقنية التفريد الكهربي بطريقة الصوديوم ديدوسيل سلفيت SDS-PAGE للبروتينات الذائبة المستخلصة من بذور طفرات الكانولا و آبائهم أظهرت بعض الحزم البروتينية المرتبطة بالمقاومة والحساسية للأمراض تحت الدراسة. وقد استخدم برنامج الحاسوب UPGMA لتوضيح التباعد الوراثي بين الطفرات وآبائهم، فتبين وجود مدى واسع في المسافات الوراثية بين كل صنف أبوي والطفرات المستنبطة منه كما أظهر ال dendrogram أن التراكيب الوراثية الأكثر مقاومة لأمراض العفن الفحمي والذبول الفيوزاريومي و تبقع الأوراق والبياض الدقيقي وقعت في نفس المجموعة وكذلك الحال بالنسبة للتراكيب الوراثية الأكثر حساسية.