#### **Genetic Analysis And Maternal Effects In Berseem Clover**

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Abstract: The objectives of this study were to estimate the genetic parameters, the six parameters, heritability, heterosis, loss of vigros and study the role of maternal effects in berseem isolated distances. Mean analysis was used to estimate the magnitude of genetic variance component, the epistatic interaction and mode of inheritance in berseem clover populations. Three crosses and their reciprocals were conducted in field and greenhouse at the Agricultural Research Station at Giza during three winter seasons. Seven generations and their reciprocals were formulated by manual hybridization and subjected to generation mean analysis plant<sup>-1</sup>, for detecting the gene effects responsible for inheritance of the plant height, number of tillers, fresh and dry weight across parents and hybrid generations. Significant differences were detected among generations viz, P1, P2, F1, F2, F3, BC1 (P1xF1) and BC<sub>2</sub> (P<sub>2</sub>xF<sub>1</sub>) for most of the studied traits in the three crosses and their reciprocals. The contributions of epitasis to the additive, dominance and interaction genetic variances were specified. Duplicate epistasis (that cause lower heteroses value) was prevailing for all the studied traits in the three crosses and their reciprocals. Epistasis made substantial contributions to each of these variance components. Low values of  $(h_n^2)$  in crosses of Miskawi male parent x Fahl female parent were observed for all traits except Giza-6 x Fahl in plant height mean (0.57).  $((h^2_b)$  was highly significant for multi-cut berseem cultivars as female parent in crosses with Fahl for Hatour with respect to plant height and fresh weight (0.90 and 0.84, respectively) and for Giza-6 with respect to average number of tillers (0.84). All crosses (Miskawi as female x Fahl as male) showed highly significant  $(H_{Bn})$ . Also reciprocals recorded the same performance for heterosis across all traits except for (Giza-6 and Hatour) with respect to plant height and for Giza-6 fresh weight. Total fresh and dry yield recorded highly depression of Giza as female parent x Fahl (68.98 and 69.18%, respectively), while Hatour as female parent x Fahl had the best performance without losses in vigor's (-10.34 and -9.36%), respectively. The (CVG) values were close to (CVP) values, suggesting low effects of environments and high genetic expression for all the studied traits.

Maternal genetic effects influenced traits throughout fresh and dry yield, including high significant direct and maternal additive genetic correlations within and between traits through cross generations. The force of maternal effects in berseem cultivars sustains the harmonious structure of population gene pool within each cultivar, enabling us to reduce isolation distance of foundation and breeder seed propagation.

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### 1. Introduction:

Egyptian clover, berseem, (*Trifolium alexandrinum*) is the most important winter forage in Egypt, India, Pakistan, Turkey and most countries of Mediterranean region. The crop is reported to be highly self-compatible requiring tripping via honey-bee visits to achieve, cross-fertility, in Egypt. Cross-ability and some morphological and cytological characters of berseem were studied by Tobgy *et al* (1974a). In a recent work, the crop proved to be self-compatible, but needs pollinating agent to result in good seed setting (Abdalla and Abd El-Naby, 2012).

The Berseem types 'Miskawi and Fahl' are two Egyptian biotypes of berseem, based on stem branching, no. of cuttings, plant vigor and seed yield. Miskawi type is a basal or crown branching type that can be cut, from four to six times during its growing season but produces low seed yield fed.<sup>-1</sup>. While Fahl type is a stem branching and is cut only once but has more seed yield compared with Miskawi. Several authors crossed Miskawi and Fahl berseem cultivars as a tool to improve forage yield and quality cultivars. Bakheit (1996) developed a new multi-foliate multi-cut strain of berseem clover by crossing a mutant of mono-cut Fahl cultivar having multi foliate leaves and a multi-cut Meskawi cultivar with trifoliate leaves.

Heterosis or hybrid vigor may be defined as the superiority of the heterozygote over the midparent or over the better parent. Fonesca and Patterson (1968) proposed the term 'heterobeltiosis' to describe the improvement of the heterozygote over the better parent. Both dominance and epistasis have been recognized as capable of producing heterotic response (Graffius, 1959).

As outlined by Kearsey and Pooni (1996), generation mean analysis is considered a useful technique in plant breeding for estimating main genetic effects (additive and dominance) and their digenic (additive x additive, additive x dominance, and dominance x dominance) interactions responsible for inheritance of quantitative traits. It helps in understanding the performance of the parents used in crosses and potential of crosses to be used either for the heterosis exploitation or pedigree selection (Sharma and Sain, 2003 and 2004). The basic work carried out by Gamble (1962) clearly indicated the role of epistatic gene actions in addition to additive and dominance types in controlling the inheritance of yield and yieldassociated traits in different crops. Besides, Cavalli (1952) reported that accuracy of gene effects increases with increasing the number of segregating generations and the number of observational plants. This analysis of generation parameters has contributed to the understanding of genetic parameters associated with many important agronomic characters, leading to gains in selection efficiency. In previous study (Abo-Feteih et al 2010)  $F_2$  hybrid generations were superior agronomically for both parents while retaining the qualities of the maternal parent. Abd El-Naby and Abo-Feteih (2012) used manual hybridization to develop two new populations, multi-branching population by crossing between Miskawi female parent x Fahl male parent and short duration population by its reciprocal.

The present investigation was conducted to determine: 1) the extent of heterosis, phenotypic and genotypic coefficients of variations, maternal effects as well as the nature of gene action involved in the inheritance of yield and some agronomic characters of three multi-cut x mono-cut berseem type crosses and their reciprocals, 2) Estimates of the six parameter technique for genetic parameters for crosses between multi-cut and mono-cut types, 3) study the existence of maternal effects in berseem clover populations to determine isolation distances of berseem clover foundation seed fields and 4) select plants for both basal and stem branching and high yield with the ability to obtain more than three cuttings during the growing season with improved plant vigor, forage and seed yield through the production of more tillers and branches in plant progenies.

### 2. Materials and Methods Plant materials:

Three multi-cut type berseem; two cultivars Helaly and Giza-6 as well as a new promising population called Hatour produced by selection for high self-fertility (Abd El-Naby, 2003 and 2009) and mono-cut type berseem, Fahl, were sown in April 2009 under the greenhouse conditions. Seven basic generations population<sup>-1</sup> were formulated by manual hybridization (Fig. 1). These generations were two parents (P<sub>1</sub>, P<sub>2</sub>), first, second and third filial generations (F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub>), first backcross BC<sub>1</sub> (P<sub>1</sub>xF<sub>1</sub>) with the 2<sup>*nd*</sup> parent backcross BC<sub>2</sub> (P<sub>2</sub> x F<sub>1</sub>) were produced. Individual selection for vigros between plants and within generations was used among crosses and their reciprocals. The experimental materials of this study were generated at Agricultural Research Station in Giza during three growing seasons (2009 – 2011).

# Methods:

The experimental materials were planted in a randomized complete block design in three replications. Each generation was planted in 4 m long plot with a between row spacing of 25 cm and within--row spacing of 10 cm, while the number of rows plot<sup>-1</sup> and the number of analyzed plants plot<sup>-1</sup> varied with generations. Observations were recorded on 10 plants chosen at random from each plot. Growth and yield characteristics were plant height, the number of basal tillers (>8cm) and fresh and dry forage yields plant<sup>-1</sup>.

The following notations for gene effects were used: [m]-mean, [a]-additive, [d]-dominance, [i]additive x additive, [j]-additive x dominance and [I]-dominance x dominance effects. The type of epistasis was determined only when dominance [d] and dominance x dominance [I] effects were significant. When these effects had the same sign, the type of epistasis was complementary (i.e. useful epistasis), while different signs indicated duplicate epistasis (Kearsey and Pooni, 1996).

## Statistical and genetic procedures:

Heterosis and statistical analysis were carried out using the inbreeding depression (%) estimated according to Burton and Brownie (2006). The Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated using the formula suggested by Dudley and Moll (1969).

The phenotypic  $(\sigma^2_P)$ , genetic  $(\sigma^2_G)$ , additive  $(\sigma_{\rm A}^2)$ , dominant  $(\sigma_{\rm D}^2)$  and environmental  $(\sigma_{\rm E}^2)$ variances and the heritability in the broad  $(h_b^2)$  and narrow (h<sup>2</sup><sub>n</sub>) senses were estimated according to Allard (1999). The heterosis estimate was based on a model similar to that initially described by Gardner and Eberhart (1966):  $H_{BP}(\%) = (F1-BP) /$ BP\*100, where:  $H_{BP}(\%)$ =heterosis relative to the high parent,  $F_1$  = hybrid mean. The loss of vigor (L.V.) was calculated based on the model described by Vencovsky and Barriga (1992): L.V. (%) =  $(MF_1-MF_2)/MF_1*100$ , where: L.V. (%) = loss of vigor, MF<sub>1</sub>=mean of F<sub>1</sub> generation, MF<sub>2</sub>=mean of F<sub>2</sub> generation. Both broad and narrow-sense heritability ( $h_b^2$  and  $h_n^2$ , respective) were calculated according to Mather procedure (Mather, 1949).

The genetic effects for each cross were estimated for the characters using the generalized weighted least square method and testing the adjustment of the model of six parameters genetic model (complete model): mean "m", additivity "a", dominance "d", additivity x additivity "i", additivity x dominance "j" and dominance x dominance "I") and three parameters (reduced model in the absence of epistasis:  $F_2$  mean "m", additivity "a" and dominance"d"), according to Mather and Jinks (1982). The significance of the genetic parameter was verified by the t test. The (A, B and C) scaling test was applied as outlined by Gamble (1962) and used to test epitasis.

# Statistical analysis of maternal effects

The 'animal model' effects were late suggested by Kruuk, (2004) and Kruuk and Hadfield (2007), to analyze data from the three-

generation breeding design and back crosses. The animal model fits genetic parameters in nature populations using a pedigree, allowing all relationships to be used as information, in order to separate genetic and environmental causes of similarity between relatives.

We initially fitted univariate models to the four traits. First, we fitted a fully specified model, including Tray as a fixed effect, direct ( $\sigma^2 Ao$ ) and maternal ( $\sigma^2_{AM}$ ) genetic effects, a maternal environmental effect ( $\sigma^2 Em$ ) and a residual component ( $\sigma^2_{E0}$ ) as random effects (adjusted Model 1), whereas we fitted a direct ( $\sigma^2 Ao$ ) and maternal ( $\sigma^2_{AM}$ ) genetic correlation across studied traits per population.



 d
 e
 f

 Figure 1. Preparing berseem florets before relocating pollen grain to emasculate florets: a) berseem inflorescence, b+c) removal of basal whorl florets, d+e) emasculation of 10 styles from each floret and f) cover the inflorescences by glycine bag and then transfer mature pollen grain from male florets to emasculated female florets after 18 to 20 hr. by brush.

#### 3. Results

#### **Performance of Hybrids and Parents**

Table (1) shows highly significant differences (P < 0.01) for all studied traits except genotypes mean plant height. Also differences among parents and crosses were significant (P< 0.01) across all studied traits. Miskawi female, and parents vs. crosses indicated significant differences (P< 0.01) over all traits. Miskawi male parents recorded significant (P<0.05) and insignificant differences for plant height mean and average of number of tillers, respectively. The interaction effects (female vs. male parents) and (parents vs. crosses) recorded high significant differences P<0.01) across all traits. Therefore, differences between parents vs. crosses (female vs. male) that reveal additive effects were significant for all traits (Table 1).

The performance of all the tested materials was good for all studied characters. However, selection for vigor plants led to improving yield through successive hybrid generations.

#### Miskawi female x Fahl male parent crosses

The performance of the hybrid generation's mean compared to their control female parent population is presented in Table (2), where a high magnitude and positive sign was found for fresh and dry yield plant<sup>-1</sup> of Hatour as female parent x Fahl male parent with total weight plant<sup>-1</sup> (819.2 and 120.08 g), respectively comparing with the other hybrids (i.e. Helaly x Fahl or Giza-6 x Fahl)

| Source of variation     | đf   |                | Mean s      | squares        |              |
|-------------------------|------|----------------|-------------|----------------|--------------|
| Source of variation     | u.1. | Plant hight cm | No. tillers | Fresh weight g | Dry weight g |
| Replications            | 3    | 11.25          | 30.05       | 7541.41        | 3282.92      |
| Genotypes               | 9    | 78.25          | 4259.58**   | 2993224.67**   | 70943.30**   |
| Parents                 | 3    | 4875.43**      | 4822.54**   | 2582793.02**   | 58629.12**   |
| Crosses                 | 5    | 2544.01**      | 3976.07**   | 3806325.93**   | 91611.04**   |
| Miskawi female crosses  | 2    | 1745.41**      | 3061.11**   | 4257979.67**   | 102561.80**  |
| Miskawi male crosses    | 2    | 122.03*        | 1.508       | 105777.71**    | 2707.90**    |
| Female vs. male crosses | 1    | 8985.17**      | 13755.14**  | 10304114.85**  | 247515.79**  |
| Parents vs. crosses     | 1    | 4553.96**      | 159013.30** | 159013.30**    | 4547.1**     |
| Error                   | 27   | 45.294         | 5.969       | 7541.41        | 192.83       |
| Error                   | 27   | 45.294         | 5.969       | 7541.41        | 192.83       |

| Table 1. Analysis of variances of Miskawi and Fahl berseem cross |
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|--|

n.s., \*and\*\* insignificant, significant and highly significant differences at the levels of probability (0.05 and 0.01, respectively).

across all generations. The maximum expression of vigor was observed for Hatour followed by Helaly x Fahl and Giza-6 x Fahl generations, revealing a large decrease when comparing the  $F_2$  and  $F_1$  generation means. This is logic and expected since the expression of heterosis in  $F_1$  will be followed

by a considerable reduction in  $F_2$  due to increases in homozygosity and decreases in heterozygosity. By contrast, Hatour with Fahl cross showed in  $F_2$ total fresh and dry weight (g) plant<sup>-1</sup> higher than  $F_1$ generation, this increase was related to the force of hybrid vigros and selection among plants.

Table 2. Means for four traits of  $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $F_3$ ,  $BC_1$  and  $BC_2$  generations (±SE) derived from three Miskawi populations as a female parent in crosses with mono-cut Fahl berseem populations with their reciprocals.

|                 |            | Plant he         | ight cm     | No. Tillers      |                 |                | Fresh weight (g) |            |              |            | Dry weight (g)    |            |  |
|-----------------|------------|------------------|-------------|------------------|-----------------|----------------|------------------|------------|--------------|------------|-------------------|------------|--|
|                 | Giza-6     | Hatour           | Helaly      | Giza-6           | Hatour          | Helaly         | Giza-6           | Hatour     | Helaly       | Giza-6     | Hatour            | Helaly     |  |
|                 |            |                  |             |                  | Miskaw          | vi female p    | arent            |            |              |            |                   |            |  |
| Miskawi female  | 75.73±0.26 | 83.95±1.07       | 72.53±0.47  | 16.01±0.17       | 37.55±0.49      | 17.97±0.34     | 539.94±17.4      | 822.4±18.4 | 577.17±12.6  | 84.48±2.71 | 128.01±2.9        | 97.18±1.9  |  |
| $\mathbf{F}_1$  | 78.06±0.45 | 90.61±0.52       | 80.766±0.64 | 8.80±0.13        | 26.13±0.32      | 15.35±0.28     | 344.91±2.70      | 994.8±10.0 | 619.59±9.2   | 54.13±0.42 | 155.12±1.6        | 110.02±1.5 |  |
| $\mathbf{F}_2$  | 77.48±0.24 | 94.33±0.44       | 86.58±0.74  | 15.42±0.45       | 24.79±0.54      | 15.24±0.32     | 582.8±8.9        | 889±18.12  | 571.7±14.6   | 91.58±1.42 | $140.59 \pm 2.04$ | 96.07±2.33 |  |
| F <sub>3</sub>  | 77.94±0.18 | 95.80±0.41       | 80.99±0.22  | $14.00 \pm 0.20$ | 30.79±0.19      | 18.55±0.18     | 487.55±6.15      | 802.8±16.1 | 526.47±7.01  | 77.53±0.98 | 120.23±1.6        | 88.91±1.7  |  |
| BC <sub>1</sub> | 80.02±0.34 | 97.35±0.82       | 77.39±0.15  | 17.31±0.23       | 24.64±0.73      | 15.08±0.73     | 451.99±10.9      | 666.2±22.6 | 551.90±24.83 | 75.93±1.86 | 112.22±2.7        | 99.2±4.3   |  |
| Mean            | 77.85      | 92.31            | 79.65       | 13.35            | 28.82           | 16.44          | 466.82           | 834.05     | 570.03       | 76.73      | 131.34            | 98.29      |  |
|                 |            |                  |             |                  | Miska           | wi male p      | arent            |            |              |            |                   |            |  |
| Fahl female     | 96.64±0.81 | 96.64±0.81       | 96.64±0.81  | $1.0 \pm 0.016$  | $1.0 \pm 0.016$ | $1.0\pm 0.016$ | 239.2±79.1       | 239.2±79.1 | 239.2±79.1   | 40.67±1.76 | 40.32±1.76        | 40.32±1.76 |  |
| $\mathbf{F}_1$  | 95.70±0.71 | 93.50±0.49       | 93.5±0.42   | 1.67±0.11        | 2.4±0.08        | $1.40\pm0.07$  | 212.2±4.14       | 368.9±4.33 | 206.0±3.44   | 33.96±0.66 | 62.9±0.55         | 32.94±0.55 |  |
| $\mathbf{F}_2$  | 98.14±0.86 | $101.7{\pm}0.87$ | 97.83±0.52  | 3.77±0.72        | 9.53±.82        | $5.17 \pm .82$ | 341.1±7.7        | 446.97±7.9 | 359.27±7.9   | 57.99±1.31 | 76.03±1.3         | 61.12±1.33 |  |
| F <sub>3</sub>  | 98.68±0.34 | 99.35±0.31       | 99.4±0.31   | 5.35±0.42        | 9.52±1.31       | 8.33±1.08      | 331.5±7.97       | 471.4±19.4 | 371.5±19.4   | 52.53±1.36 | 75.4±3.31         | 63.14±3.30 |  |
| BC <sub>2</sub> | 94.86±0.59 | 97.35±0.82       | 105.3±1.29  | 3.42±0.23        | 7.67±1.60       | 7.08±2.61      | 374.5±20.73      | 632.1±22.6 | 405.5±36.5   | 56.96±3.52 | 103.5±4.75        | 74.86±4.58 |  |
| Mean            | 97.73      | 96.50            | 98.1        | 3.41             | 5.565           | 4.60           | 287.14           | 443.49     | 323.3        | 48.22      | 72.01             | 54.477     |  |

Table 3-a. Estimates of some genetic parameters from crosses of three female parents multi-cut berseem clover populations with Fahl (mono-cut cultivar) as male parent population.

| Genetic                     | Р       | lant hight o | cm       | No. of tillers |          |          | Fresh weight g |         |        | Dry weight g |         |        |
|-----------------------------|---------|--------------|----------|----------------|----------|----------|----------------|---------|--------|--------------|---------|--------|
| parameter                   | Giza-6  | Hatour       | Helaly   | Giza-6         | Hatour   | Helaly   | Giza-6         | Hatour  | Helaly | Giza-6       | Hatour  | Helaly |
| Narrow sense h <sup>2</sup> | 0.41    | 0.90**       | 0.52     | 0.83**         | 0.47     | 0.09     | 0.71*          | 0.66    | 0.68   | 0.19         | 0.62    | 0.31   |
| Broad sense h <sup>2</sup>  | 0.43    | 0.91**       | 0.53     | 0.84**         | 0.79**   | 0.78*    | 0.72*          | 0.84**  | 0.74*  | 0.71*        | 0.77*   | 0.73*  |
| Genetic Adv%                | 22.89   | 11.08        | 70.23    | 85.23          | 30.36    | 6.20     | 30.79          | 13.68   | 52.18  | 8.17         | 62.68   | 37.33  |
| Heterosis Bp                | 3.07 ** | -6.23**      | -16.98** | -45.04**       | -30.42** | -14.60** | -36.12**       | 20.97** | 7.35** | -35.92**     | 20.66** | 6.33   |
| Loss vigor (LS)             | 0.74    | -4.11**      | -7.20**  | -75.2**        | 5.13**   | 0.72     | -68.97**       | 10.64*  | 7.73   | -69.19**     | 9.37    | 12.68* |
| P.C.V.                      | 8.39    | 5.89         | 12.20    | 22.77          | 30.93    | 41.96    | 20.94          | 11.00   | 36.04  | 21.99        | 29.17   | 36.28  |
| G.C.V.                      | 5.19    | 3.92         | 7.86     | 18.18          | 23.47    | 32.22    | 17.38          | 8.66    | 26.37  | 14.13        | 21.73   | 26.39  |
| E.C.V.                      | 3.21    | 1.97         | 4.34     | 4.58           | 7.46     | 9.74     | 6.75           | 2.34    | 9.67   | 6.86         | 7.44    | 9.89   |

\*and\*\* significant and high significant differences at the levels of probability (0,05 and 0.01, respectively).

 Table 3-b. Estimates of some genetic parameters of the reciprocal crosses between the three male parent (multi-cut)

 berseem clover populations with Fahl (mono-cut cultivar) as female parent population.

| Genetic                     | I       | Plant hight | cm       | No. tillers |           |           | Fresh weight g |          |          | Dry weight g |          |          |
|-----------------------------|---------|-------------|----------|-------------|-----------|-----------|----------------|----------|----------|--------------|----------|----------|
| parameter RC                | Giza-6  | Hatour      | Helaly   | Giza-6      | Hatour    | Helaly    | Giza-6         | Hatour   | Helaly   | Giza-6       | Hatour   | Helaly   |
| Narrow sense h <sup>2</sup> | 0.57    | 0.72*       | 0.52     | 0.08        | 0.07      | 0.06      | 0.29           | 0.09     | 0.08     | 0.17         | 0.42     | 0.08     |
| Broad sense h <sup>2</sup>  | 0.78*   | 0.92**      | 0.53     | 0.61        | 0.96**    | 0.99**    | 0.39           | 0.51     | 0.68     | 0.47         | 0.67     | 0.71*    |
| Genetic Adv%                | 58.00   | 19.90       | 69.03    | -82.80      | -18.90    | -96.36    | 33.03          | -60.60   | -58.89   | 9.43         | 24.09    | -65.8    |
| Heterosis Bp                | -0.97   | 0.32        | -16.40** | -89.54**    | -95.27**  | -92.21**  | -0.65          | -63.62** | -40.91** | -16.5**      | -62.75** | -18.31** |
| Loss vigor (LS)             | -2.55** | -8.77**     | -4.63**  | -125.7**    | -297.08** | -269.29** | -60.74**       | -21.16   | -74.40** | -70.73**     | -20.87   | -85.55** |
| P.C.V.                      | 20.68   | 11.8        | 12.24    | 71.70       | 61.60     | 73.00     | 27.31          | 27.69    | 30.93    | 27.32        | 28.46    | 30.92    |
| G.C.V.                      | 16.34   | 8.56        | 7.87     | 55.25       | 48.80     | 57.73     | 15.00          | 16.12    | 20.32    | 15.83        | 19.45    | 20.79    |
| E.C.V.                      | 4.34    | 3.19        | 4.36     | 16.45       | 12.80     | 15.32     | 12.30          | 11.98    | 10.61    | 11.48        | 9.01     | 10.20    |

\*and\*\* significant and high significant differences at the levels of probability (0,05 and 0.01, respectively).

### Miskawi male x Fahl female parent crosses

Some of the characteristics under study showed more variation among generation means. With a few exceptions, the trend of decreased  $F_1$ performance was greater than the top parents for all traits. Both F<sub>1</sub> and F<sub>2</sub> means were close to superior parents for all crosses. F1 mean was lower than the higher parent but selected F<sub>1</sub> plants produce greater means of  $F_2$  and  $F_3$  generations. All the generation means for fresh and dry weight were close to the inferior parent. Both BC generation means were greater than the superior parent for all the traits, except the BC<sub>1</sub> plant height of Giza-6 as male parent x F<sub>1</sub> generation (Giza-6 male x Fahl female cross) which was close to the superior parent (Table 2). F<sub>2</sub> generation of Hatour female parent cross had the best performance across all traits compared with the other female crosses, with Fah, for plant height mean (94.33 cm), average number of tillers (24.79), total fresh weight (889) and total dry weight (140.59), respectively. Hatour x Fahl crosses either as female or male parent noted the best performance across all generations and crosses of the studied traits.

#### Genetic parameters Heritability

For almost all traits, the heritability was calculated using the female and male variances represented in Table (3  $_{a \text{ and }b}$ ). Table 3-a shows that narrow sense heritability ( $h^2_n$ ) had high significant effects for Miskawi female hybrids with Fahl in plant height mean and average number of tillers (0.90 and 0.83) of Hatour and Giza-6 crosses. Significant effects were recorded for Giza-6 x Fahl cross of total fresh weight plant<sup>-1</sup> (0.70) Table (3-a). Broad sense heritability ( $h^2_b$ ) was highly significant for Hatour x Fahl with respect to plant height mean and total fresh weight plant<sup>-1</sup> (0.91 and 0.84, respectively) and for average number of tillers (0.84) of Giza-6 male parent x Fahl hybrid.

Low values of  $(h_n^2)$  in crosses of Miskawi male parent x Fahl female parent were observed for all traits except Giza-6 x Fahl and Hatour x Fahl in plant height mean (0.57 and 0.72) (Table 3-b).  $(h_b^2)$ was highly significant for Hatour male x Fahl female parent with respect to plant height and average number of tillers (0.92 and 0.96, respectively) and for Helaly x Fahl tillers mean (0.99). Significant  $(h_b^2)$  effects were noticed for Giza-6 male parent x Fahl for plant height mean and Helaly male parent x Fahl for total dry weight and plant height mean (0.78 and 0.71), respectively.

### Heterosis

All crosses over all traits in Table (3-a) showed highly significant heterosis against better parent (i.e. heterobeltiosis) except Helaly as female parent with Fahl for dry weight. Beside Table (3-b) recorded the same highly significant heterosis

across all crosses and traits except for (Giza-6 and Hatour as male parents x Fahl as female parent) hybrids with respect to plant height Genetically estimates of Miskawi as female parent hybrids with Fahl Table (3-a) shows that Hatour as female parent x Fahl hybrid noted the best heterosis (H<sub>Bp</sub>) over all hybrids for total fresh and dry weight (20.97 and 20.66%, respectively). Table (3-b) shows lower heterosis (H<sub>Bp</sub> %) from to the better parent for average number of tiller (-95.27 and -92.21%) for Hatour and Helaly male parents crosses hybrids. Hatour male parent with Fahl as female parent cross was the lowest H<sub>Bp</sub> % for plant height mean (-0.32%).

Also, negative signs were observed for all the multi-cut cultivars (Giza-6, Hatour and Helaly) hybrids with Fahl for all traits except for total fresh and dry weight plant<sup>-1</sup> in two out of the three crosses (Table 3-a). Hatour plant height in reciprocal crosses showed positive sign of heterosis (H<sub>Bp</sub>%). (Table 3-b) The differences in heterosis magnitudes might be due to genetic variability of the parents and non-allelic interactions, which can either lead to increase or decrease the expression of heterosis. Even in the absence of epistasis, multiple alleles at a locus could lead to either positive or negative heterosis (Cress, 1966).

# Loss of vigor

Loss of vigor measures the reduction in performance of the F<sub>2</sub> generation due to loss of vigor in female and male Miskawi hybrids with Fahl. The results shows highly significant positive differences between F1 and F2 means of Miskawi multi-cut female parents with Fahl except for Giza-6 plant height (Table 3-a). Hatour female hybrid with Fahl recorded highly significant effects (P<0.01) with negative sign across all traits except for plant height. Helaly female hybrid with Fahl recorded positive signs across all traits except plant height. Total fresh and dry yield recorded highly depression of Giza hybrid with Fahl (68.98 and 69.18%), respectively, while Hatour hybrid had the best performance without losses in vigor's % (-10.34 and -9.36%), respectively.

Positive effects for both heterosis and loss of vigor across all Miskawi cultivars as female or male parents seem logic since the expression of heterosis in  $F_1$  was followed by considerable reduction in the  $F_2$  performance. Also, reduction in heterozygosity and consequently in the values of non-additive genetic components due to inbreeding logically the major cause of loss of vigor. These results are in agreement with those obtained by Abul-Naas *et al* (1993) and Sharma *et al* (2004).

# Component of variance and related genetic parameters

Component of variance and genetic parameters for different growth and yield characters are presented (Tables 3 <sub>a and b</sub>). Phenotypic expression of the characters is a result

of interaction between genotypes and environment. While, genotypic coefficient of variation (GCV) measures the range of variability in crop and both enables to compare the amount of variability present in different characters. The phenotypic coefficient of variation (PCV) estimates was higher than genotypic coefficients of variation (GCV) for all the characters studied. It could be indicated from the results that substantial influence of environment in the expression of these characters with both maternal and paternal Miskawi berseem crosses were shown.

High GCVs and PCVs were observed for the average number of tillers (in both female and male Miskawi crosses with Fahl). These findings were reported earlier by (Gill, *et al* 1977 and Memon, 2010). Abdalla and Abd El-Naby (2013) found that the (CVG) was close to (CVP) and suggested low effects of environments and high genetic expression for all studied traits.

Results in our study revealed that berseem clover heritability recorded presence of selffertility as reported by (Finn, 1964; Chowdhry *et al.*, 1966; Beri *et al* 1985a and b; Dixit *et al* 1989 and Abd El-Naby, 2003).

### Hybridization potentials

# 1) Miskawi as female parents in crosses with Fahl berseem:

# a) Scaling Tests

The results of scaling tests (A, B and C) are presented in Table (4-a) indicated highly significant differences (P<0.01) for all studied traits in the three female parent's crosses except for total fresh and dry weight plant<sup>-1</sup> for scale A and B of Helaly population. Also, cross with Giza -6 population showed insignificant differences for scale A in total fresh weight plant<sup>-1</sup>. On the other hand scale A recorded highly significant negative differences for average number of tillers, total fresh and dry weight plant<sup>-1</sup> for Hatour and Helaly crosses, respectively. This result is considered good indicator for the percentage of epistasis in the four traits.

### b) Gene action and six parameter

Different types of gene effects are presented (Table 4-a). The  $F_2$  estimated mean effect parameter (m) was found to be highly significant for number of tillers (24.79), total fresh and dry weight plant<sup>-1</sup> (889.0 and 140.59 g), respectively of Hatour female parent cross with Fahl. Fresh and dry weight of the  $F_2$  generation of Giza-6 and Helaly female crosses noted similar results. Initially, it is clear that all studied traits were quantitatively inherited.

Estimates of the six parameter model revealed the significance of both additive and nonadditive gene effects for most traits. No significant differences of additive (a) gene effect were observed for total fresh weight plant<sup>-1</sup> of Hatour and Helaly as female parent with Fahl as the male parent. Also, for plant height mean (Giza-6 and Helaly with Fahl) and for total fresh weight plant<sup>-1</sup> (Helaly x Fahl) the relative contribution of dominance gene effects was higher than that of additive gene effects (Table 4-a), which was in conformity with Dahiya and Satija (1978), Dahiya and Waldia (1982), Singh and Singh (1984) and Aher and Dahat (1999).

Additive (a) gene effects were found to be highly significant with positive sign for all characters over the three crosses except for plant height across all crosses and average number of tillers of Giza-6 hybrid. This result suggested the potential improvement of these traits by using individual plant selection. These results are in close agreement with those of Saleem *et al* (2005). In general, greater importance of additive gene effects for the expression of most of the above studied attributes in the three Miskawi female parents crosses with Fahl berseem (Table 4-a).

Dominance effects (d) indicated highly significant differences among all crosses and traits except for Giza-6 hybrid in total fresh and dry weight plant<sup>-1</sup> and for Hatour cross in total dry weight plant<sup>-1</sup>. Dominance effects (d) had a negative (-)sign over all traits and crosses except for plant height mean of Giza-6 and Helaly hybrids, fresh weight cross and dry weight over all crosses were having positive (+) signs.

Among the epistatic interactions, the additive x additive (i) effects were comparatively higher for plant height mean over all Miskawi female parent crosses with Fahl male parent and for Helaly total dry weight. In contrast, the magnitude of dominance x dominance (I) was greater than (a) and (j) types but it had reducing (-) effect for the expression of means of plant height across all and total fresh and dry weights plant<sup>-1</sup> of Helaly female parent hybrid, whereas, increasing (+) effect was noticed for number of tillers of all Miskawi female populations crosses with Fahl and for total fresh and dry weight plant<sup>-1</sup> of Giza-6 and Hatour hybrids with Fahl, respectively (Table 4-a).

# 2- Miskawi as male parent in crosses with Fahl berseem

### a) Scaling Tests

Table (4-b) shows crosses of Fahl as female parent x Miskawi as male parent, scaling testes and six parameter analyses for gene action.

Scaling tests A, B and C indicated highly significant differences among all crosses except plant height mean of Giza-6 hybrid with Fahl, Hatour hybrid with Fahl for scale (B) and Helaly hybrid with Fahl in average of tillers number for scale (C). Whereas, scales recorded (-) sing in plant height mean of Giza-6 and Helaly hybrids with Fahl for scale (A), Giza-6 and Hatour hybrids with Fahl in plant height mean average number of tiller plant<sup>-1</sup> for scale (B) and all crosses in average number of tillers plant<sup>-1</sup> for scaling test (C) across all Fahl female parent crosses (Table 4-b).

## b) Gene action and six parameter

Highly significant differences (P<0.01) across all crosses and traits analyzed by six parameter method are shows (Table 4-b). Hatour x Fahl F<sub>2</sub> generation showed best performance across all traits compared with other Miskawi male parent crosses. Additive (a), dominance (d) and their interactions (i, j and I) of gene effects were highly significant over all crosses and agronomical characters under study. Additive (a) showed positive sign across all crosses and traits except for plant height mean plant<sup>-1</sup> over all crosses and for average number of tillers of Hatour hybrid. The relative contribution of dominance (d) gene effect was higher than that of additive (a) gene effect except for Giza-6 and Hatour hybrids with Fahl in mean of plant height plant<sup>-1</sup> and Hatour hybrid with Fahl in average number of tillers plant<sup>-1</sup>. It seems that the negative or positive signs for additive effects depend on which parent is chosen as  $P_1$ (Cukadar-Olmedo and Miller 1997; Edwards et al 1975).

The estimates of (d) effects were highly significant for all the studied traits in the three crosses and their reciprocals, indicating the importance of dominance gene effects in inheritance of these traits. These results are in harmony with those reported by Abd Elkader (2006) and El-Ameen (2008).

As shown in Table (4-b) dominance x dominance (I) epitatic types were highly significant and positive for Giza-6 and Helaly hybrids with Fahl in plant height mean and Hatour hybrid with Fahl in average number of tillers plant<sup>-1</sup>, while it was significant and negative for the other studied traits over all crosses. Highly significant positive additive x dominance (j) was found over all crosses except for Giza-6 x Fahl in total fresh and dry weight plant<sup>-1</sup> and Helaly x Fahl hybrid in average number of tillers plant<sup>-1</sup>. Additive x dominance (j) was higher than (i) and (I) for Hatour hybrid for mean plant height and average number of tillers as well as for Giza-6 X Fahl for total fresh and dry weight plant<sup>-1</sup>. These results show that both additive and dominance as well as one or more of the three types of epistasis, i, e., (i), (j) and (I) were important in the genetic system controlling most studied traits. The average number of tillers, total fresh and dry weights plant<sup>-1</sup> which were distinguished by a higher contribution of the additive effects compared to the dominant component in the genetic control of the character (Tables 4 a and b). It has been generally assumed that epistasis were an important portion of the genetic variances in berseem clover populations to explain allelic and non-allelic gene action.

Duplicate epistasis (Tables  $4_{a \text{ and } b}$ ) was observed, as revealed by difference in signs of (d)

and (I) in crosses which exhibited significant epistasis. These findings illustrated that duplicate epistasis (that cause lower heteroses value) was prevailing for all studied traits in the three crosses and their reciprocals. This indicated that duplicate epistasis was greater and more important for all studied traits. These results indicated that complementary type of epistasis (that leads to higher heterosis) was found for Hatour and Giza-6 female crosses with Fahl as male parent for mean plant height and total dry yield, respectively, in Table (4-a) and for Hatour and Helaly male crosses with Fahl as female parent for mean plant height (Table 4-b).

As observed in the model adjustments for the three target crosses and characters, with their reciprocals (Tables 4  $_{a \text{ and } b}$ ). This can be explained by the fact that additive effects represent the sum of individual additive, dominance gene effects and their values can be positive or negative. Estimates may turn out to be non-significant, even when each of the genes involved, individually, shows substantial additive. This could occur in cases where the genes act in opposing directions with hybridization cancelling their effects (Mather and Jinks, 1982; Saleem *et al.* 2005).

Epistatic effects were observed in the three crosses (Table 4-a) tested for Miskawi as female parents. The epistatic effects (i) always seemed superior to one or both interactions (j) and (I), which is favorable for the breeder of selfpollinating crops and under self-pollination in berseem. Mean plant height was completely dominant. On the other hand, Miskawi as male parent crosses with Fahl berseem in Table (4-b) reported epistatic effects in all tested traits. The epistatic effects i (additive x additive) and j (additive x dominance) were always higher than the interaction of I (dominance x dominance), also showing the importance of an analysis considering the complete additive-dominant-epistatic model, for quantitative characters (Cockerham, 1954).

The epistatic effects of dominance x dominance "I" can be a fixable component of the genetic variation and easily explored for some characters through hybridizations and selection procedures (Saleem *et al* 2005). Positive sign was found for Helaly cross with Fahl with respect to dry weight for both (i) and (j) epestatic types (Tables 4- a and b). Also, positive signs were found in the average number of tillers (Giza-6 and Helaly crosses with Fahl) and in total fresh and dry weight plant<sup>-1</sup> (Hatour cross with Fahl) (Table 4-b).

The signs associated with estimates of (i), (j) and (l) types of epistasis indicate the direction in which the gene effect influence the mean of the population. For (i) and (j), the sign also provides information on the association or dispersion of genes in the parents (Mather and Jinks, 1982). With two exceptions, all the other signs of (i) and (j)

types of detected epistasis were negative in the present work. Also, a negative sign for any of the two parameters suggests an interaction between increasing and decreasing alleles, thus providing evidence for some level of dispersion in the female parents. A negative sign for each of these two parameters suggests that it should be possible to improve the level of the corresponding traits. The estimates of (1) were opposite to that of (d) in both crosses, indicating duplicate epistasis (i.e. unfavorable epistasis) (Tables 4 a and b).

The higher estimates of dominance variance as compared to additive variance for all the four characters of Miskawi as male parent with Fahl were probably due to predominance of non-additive gene action suggesting the selection of parents is a prime requisite for any successful breeding program especially for exploiting heterosis. Results of genetic parameters, gene action and six populations in our present study are in harmony and agreed with Abo-Feteih *et al* (2010) and Abd El-Naby and Abo-Feteih (2012).

| Table 4-a. Estimation of Miskawi as female parent genes effect for the crosses with Fahl berseem via six p | arameter analys | is for for | ur agronomic |
|--|-----------------|------------|--------------|
| characters.  |                 |            |              |

| Six                  | 1             | Plant hight   | c m          |              | Notillers      |              | Fre          | sh weight /g   |                |                    | Dry weight/        | g             |
|----------------------|---------------|---------------|--------------|--------------|----------------|--------------|--------------|----------------|----------------|--------------------|--------------------|---------------|
| narameter            |               |               |              |              |                | Sci          | alingtest    |                |                |                    |                    |               |
| parameter            | Giza-6        | Hatour        | Helaly       | Giza-6       | Hatour         | Helaly       | Giza-6       | Hatour         | Helaly         | Giza-6             | Hatour             | Helaly        |
| A                    | 6.24±0.96**   | 20.15±2.9**   | 1.49±1.1**   | 3.19±.37**   | -14.62±1.1**   | -3.03±.63**  | 19.13±28.13  | -484.9±7.9**   | -92.96±28.74** | 13.25±3.3**        | -58.68±4.74**      | 9.44±6.53     |
| В                    | 19.53±1.1**   | 22.68±0.96**  | 33.21±2.99** | -2.95±1.92** | -2.16±0.99**   | -2.2±.66**   | 71.97±42.9** | 32.25±16.46**  | 21.93±27.0     | 18.27±7.31**       | 19.49±6.49**       | 11.38±7.70    |
| с                    | -17.66±2.1**  | 16.02±1.89**  | 15.63±4.07** | 7.99±1.04**  | 8.11±3.31**    | 11.29±1.45** | 864.42±39**  | 506.73±29.19** | 231.11±63.4**  | 133.26±6.3**       | 26.84±9.4**        | 31.74±10.06** |
|                      | Six parameter |               |              |              |                |              |              |                |                |                    |                    |               |
| Mean(m)              | 77.48±.45**   | 94.33±39**    | 86.58±.74**  | 15.42±0.45** | 24.79±.54**    | 15.24±32**   | 582.8±8.9**  | 889±18.12**    | 571.7±14.6**   | 91.58±1.42**       | 140.59±2.04**      | 96.09±2.33**  |
| Additive (a)         | -16.62±.68**  | -7.67±0.4**   | -27.82±.91** | 8.0±0.70**   | -18.07±2.7**   | 8.07±027**   | 1205±31.3**  | 34.04±31.09    | 111.52±16.73   | 19.57±5.3**        | 4.76±2.8           | 34.96±3.99**  |
| Dominance(d)         | 35.77±2.06**  | -69.32±1.84** | 15.50±3.5**  | -10.78±2.3** | -34 94 9±5.9** | -10.67±15**  | -30.9±73.02  | -492.69±96.6** | 106939±106.5** | 13.66±10.11        | -52 31±10.93       | 59.84±12.42** |
| aa(i)                | 43.43±2.17**  | 27.45±1.77**  | 19.24±3.4**  | -16.7±2.29** | -34.72#5.8**   | -16.52±1.5** | -1287±72**   | -959.38±95.5** | -294.9±59.2**  | -<br>197,4±12,07** | -<br>123.0±10.67** | 9.08±12.26    |
| ad (j)               | -6.64±0.61**  | -1.07±0.4**   | -15.85±1.0** | -0.49±0.72   | -36.34±2.7**   | -0.42±.41**  | -30.9=33.4   | -258.6±32.88** | 65.12±105.8**  | -2.5±4.53          | -39.09±3.86**      | 28.03±4.21**  |
| dd (1)               | -69.20±3.77** | -70.91±5.9**  | -53.76±4.9** | 21.97±3.4**  | 6108±11**      | 21.8±10.1**  | 664.2±132**  | 1412.8±146 9** | -373.18±44.9** | 70.22±22.4**       | 162.7±16.69**      | -87.90±18.9** |
| Type of<br>epistatis | 1.39          | 0.60          | 1.64         | 4.13         | 2.5            | 3.25         | 1.36         | 1.87           | 1.61           | -2.97              | 0.54               | 2.86          |

\*au d\*\* significant and high significant differences at the levels of probability (0.05 and 0.01, respectively).

Table 4-b. Estimation of Miskawi as male parent genes effect for crosses with Fahl berseem via six parameter analysis for four agronomic characters.

| <b>C N ( ( ( ( ( ( ( ( ( (</b> | P            | lant hight me | an cm         | Ave          | rage no. of t | illers       | To            | tal fresh weig | htg            | Tot           | al dry weigh  | tg            |  |  |
|--------------------------------|--------------|---------------|---------------|--------------|---------------|--------------|---------------|----------------|----------------|---------------|---------------|---------------|--|--|
| Scalig fest                    |              |               |               |              |               | Scali        | ng test       |                |                |               |               |               |  |  |
|                                | Giz a-6      | Hatour        | Helaly        | Giza-6       | Hatour        | Helaly       | Giza-6        | Hatour         | Helaly         | Giza-6        | Hatour        | Helaly        |  |  |
| A                              | -2.61±2.3    | 27.68±1.68**  | -22.63±.1.3** | 25.33±.31**  | 22.2±1.7**    | 11.75±.3.3** | 211.5±21.1*   | 727.13±30.8**  | 437.6±39.2**   | 38.1±5.17**   | 136.44±3.89** | 76.47±8.1**   |  |  |
| В                              | -11.39±1.1** | -1.11±1.34    | 57.28±1.1**   | -10.84±.92** | -27.5±1.7**   | 10.91±99**   | 151.8±36.6**  | 211.22±58.3**  | 550.7±18.8**   | 33.6±5.8**    | 39.24±3.7**   | 132.33±5.8**  |  |  |
| с                              | 28.92±6**    | 30.83±3.83**  | 13.45±3.4**   | -5.28±1.85** | -3.97±3.2     | -1.09±3.28   | 160.9±38.7*   | 130.61±38.6**  | 439.8±36.3**   | 38.9±6.0**    | 39.43±7.2**   | 83.8±6.1**    |  |  |
|                                | 1            | Sixparameter  |               |              |               |              |               |                |                |               |               |               |  |  |
| Mean (m)                       | 98.18±.1.4** | 101.7±.87**   | 86.05±0.74**  | 3.77±.45**   | 9.53±.82**    | 5.17±.82**   | 341.1±6.6**   | 446.97±7.9**   | 359.27±7.9**   | 57.99±1.1**   | 75.98±1.3**   | 61.12±1.33**  |  |  |
| Additive(a)                    | 14.84±.1.1** | 7.68±1.25**   | -27.91±.56**  | 10.57±.47**  | 6.56±1.1**    | -8.07±.1.7** | -120.5±17.5** | -34.0±31.1     | -111.12±20**   | -19.57±3.3**  | 4.76±1.8**    | -34.36±3.99** |  |  |
| Dominance(d)                   | -33.44±6.2** | -101.15±4.4** | 17.39±3.2**   | 12.96±2.1**  | -18.8±3.9**   | 15.67±4.7**  | 804.22±45.6** | 575.89±70.6**  | 21017±51.6**   | 130.4±8.3**   | 98.76±7.1**   | 110.4±9.7**   |  |  |
| aa(i)                          | 42.95±6.1**  | -2.04±4.3**   | 21.18±3.18**  | 19.79±2.1**  | -1.31±3.9     | 23.75±4.7**  | -342.4±43.8** | 808.75±69.7**  | 547.4±50.8**   | -58.2±8.1**   | 135.25±7.1**  | 125.7±9.6**   |  |  |
| ad(j)                          | -4.39±1.1**  | 13.28±1.4**   | -39.96±.777** | 18.08±.47**  | 24.8±1.1**    | 0.42±.1.71   | 29.4±21.0     | 256.9±32.9**   | -57.31±21.5**  | 2.9±3.8       | 48.60±2.2**   | -28.4±4.21**  |  |  |
| dd(1)                          | 56.97±7.7**  | -26.75±6.4**  | 55.81±4.1**   | -34.25±2.6** | 6.59±5.69     | -46.42±7.6** | -565.7±78.9** | -1753.1±130**  | -1534.1±87.7** | -106.6±14.8** | 310.9±10.2**  | -335.5±17.1** |  |  |
| Type of                        | 0.63         | 119           | 1.60          | 1.03         | -2.12         | 1.91         | 0.82          | -1.11          | 1.52           | 3.01          | 20.20         | -1.54         |  |  |
| epistatis                      |              |               |               |              |               |              |               |                |                |               |               |               |  |  |

\*\* high significant differences at the levels of probability (0.01).

# Estimate direct (parental) and indirect (maternal) effects

Highly significant differences (P < 0.01) among generations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BC_1$  and  $BC_2$ ) over all studied traits were obtained (Table 5-a). Direct effect was estimated as the effect of parents in cross and their shares in  $F_1$  generation to calculate the parental effects. Also, maternal effect was estimated as the effects of  $F_2$  segregations,  $BC_1$ and  $BC_2$  (cytoplasm effects). Differences among direct and maternal effects were highly significance (P< 0.01) for all traits and over crosses. There were significant differences among (P<sub>1</sub> vs. P<sub>2</sub>) and (BC<sub>1</sub> vs. BC<sub>2</sub>) for all traits over crosses. Insignificant  $F_1$ vs. (P<sub>1</sub>+P<sub>2</sub>)/2 interaction effects were found for Helaly and Hatour crosses of plant height mean plant<sup>-1</sup> and total fresh and dry yield plant<sup>-1</sup> of Giza-6 cross (Table 5-a). None of the characters showed insignificant  $F_2$  vs. (BC<sub>1</sub>+BC<sub>2</sub>)/2 interactions over reciprocals, except total dry weight plant<sup>-1</sup> of Helaly as male parent with Fahl. Direct vs. maternal recorded highly significant differences across all traits except for Giza-6 in total fresh and dry weight plant<sup>-1</sup>. The same performances of generation as significances were observed in Table (5-b), direct effects, ( $P_1$  vs.  $P_2$ ), maternal effects and (BC<sub>1</sub> vs. BC<sub>2</sub>). Direct vs. maternal effects noted highly significant differences for all traits and reciprocals except Giza-6 male parent cross for plant height and Helaly male parent cross for number of tillers.

| Table 5-a. Analysis of variance for four traits in six populations P1, P2, F1, F2, BC1 and BC2 derived from | om  |
|---|-----|
| crosses of the three multi-cut Miskawi as a female parent x mono-cut Fahl cultivar as male pare             | nt. |

|   |      | Mean squares |              |           |             |          |           |                |            |            |              |           |           |
|---|------|--------------|--------------|-----------|-------------|----------|-----------|----------------|------------|------------|--------------|-----------|-----------|
| Source of<br>variation                          | d.f. | Р            | lant hight c | m         | No. tillers |          |           | Fresh weight g |            |            | Dry weight g |           |           |
| vunution  |      | Giza-6       | Helaly       | Hatour    | Giza-6      | Helaly   | Hatour    | Giza-6         | Helaly     | Hatour     | Giza-6       | Helaly    | Hatour    |
| Replications                                    | 3    | 50.60        | 64.29        | 23.52     | 2.89        | 23.10    | 34.82     | 4773.76        | 13831.10   | 16706.20   | 140.22       | 344.94    | 449.68    |
| Generation                                      | 5    | 310.28**     | 580.52**     | 196.16**  | 137.64**    | 169.99** | 430.86**  | 70919.16**     | 87756.24** | 285253.2** | 1583.96**    | 2865.91** | 6455.04** |
| Direct effects                                  | 2    | 113.49**     | 491.49**     | 113.49**  | 892.03**    | 333.85** | 892.04**  | 629101.1**     | 154936.5** | 629101.1** | 14402.2**    | 3628.89** | 14402.2** |
| $\mathbf{P}_1$ vs. $\mathbf{P}_2$               | 1    | 702.71**     | 962.92**     | 221.77*   | 450.71**    | 576.05** | 1409.53** | 183333**       | 24417.1*   | 677418.9** | 3899.9**     | 384.47*   | 15380.1** |
| $F_1$ vs. $(P_1+P_2)/2$                         | 1    | 99.32**      | 15.036       | 6.704     | 0.173**     | 68.74*   | 168.81**  | 3810.6         | 214091.8** | 306995.4   | 136.71       | 5154.79** | 7067.9    |
| Indirect effects                                | 2    | 352.56**     | 812.05**     | 121.736** | 116.91**    | 87.84**  | 199.84**  | 63191.36**     | 20495.9**  | 77853.7**  | 1245.55**    | 1258.8**  | 1283.2**  |
| BC <sub>1</sub> vs. BC <sub>2</sub>             | 1    | 440.61**     | 1547.9**     | 117.79**  | 223.81**    | 130.13** | 238.59**  | 29023.6**      | 26497.2**  | 2315.4     | 765.89**     | 2501.36** | 45.296    |
| $F_2 \underset{BC2}{\text{vs.}} (BC_1 + BC2)/2$ | 1    | 198.37**     | 57.11**      | 94.255**  | 7.505*      | 34.15**  | 62.739**  | 73019.3**      | 10871.1*   | 115044.0** | 1293.9**     | 12.226    | 1890.8**  |
| Direct vs.<br>indirect                          | 1    | 1346.5**     | 295.5**      | 48.019**  | 39798.9**   | 6.562*   | 48.019**  | 3.429          | 87916.19** | 12356.5**  | 11.096       | 4554.12** | 904.32**  |
| Error   | 15   | 10.709       | 12.74        | 6.972     | 1.547       | 2.874    | 5.152     | 835.27         | 1681.61    | 1386.62    | 22.39        | 43.064    | 39.199    |

\*and\*\* significant and high significant differences at the levels of probability (0.05 and 0.01, respectively).

Table 5-b. Analysis of variance for four traits in six populations P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub> derived from the reciprocal crosses between the three multi-cut Miskawi as a male parent x mono-cut Fahl as female parent.

|                                     |      |          | Mean squares |           |             |          |          |                |            |            |              |           |           |  |
|-------------------------------------|------|----------|--------------|-----------|-------------|----------|----------|----------------|------------|------------|--------------|-----------|-----------|--|
| Source of<br>variation              | d.f. | Р        | lant hight c | m         | No. tillers |          |          | Fresh weight g |            |            | Dry weight g |           |           |  |
| (unimition                          |      | Giza-6   | Helaly       | Hatour    | Giza-6      | Helaly   | Hatour   | Giza-6         | Helaly     | Hatour     | Giza-6       | Helaly    | Hatour    |  |
| Replications                        | 3    | 92.69    | 28.78        | 35.75     | 1.66        | 4.141    | 38.87    | 3784.32        | 3214.93    | 10856.92   | 104.49       | 79.98     | 298.34    |  |
| Generation                          | 5    | 356.29** | 395.51**     | 528.13**  | 171.51**    | 416.04** | 339.18** | 63101.8**      | 207578.7** | 126350.1** | 1534.8**     | 5772.59** | 3838.79** |  |
| Direct effects                      | 2    | 500.80** | 134.57**     | 723.24**  | 287.5**     | 925.74** | 367.29** | 133111.2**     | 477181.3** | 12249.7**  | 3028.2**     | 1118.2**  | 192.47**  |  |
| $\mathbf{P}_1$ vs. $\mathbf{P}_2$   | 1    | 702.71** | 221.8**      | 962.92**  | 450.7**     | 1409.5** | 576.04** | 183333.2**     | 677418.9** | 24417.2**  | 3899.9**     | 15380.1** | 384.48**  |  |
| $F_1$ vs. $(P_1+P_2)/2$             | 1    | 224.08** | 35.57*       | 362.74**  | 93.366**    | 331.53** | 118.81** | 62165.1**      | 207706.2** | 61.771     | 1617.3**     | 5246.9**  | 0.357     |  |
| Indirect effects                    | 2    | 373.08** | 828.78**     | 59.35**   | 138.18**    | 112.16** | 257.03** | 19409.50**     | 38885.4**  | 55665.4**  | 512.269**    | 2571.2**  | 1551.04** |  |
| BC <sub>1</sub> vs. BC <sub>2</sub> | 1    | 440.61*  | 1547.9*      | 117.79**  | 223.8**     | 130.13*  | 296.33** | 29023.6**      | 26497.2**  | 2315.40    | 765.89**     | 2501.36*  | 45.29     |  |
| $B F_2 vs. (BC_1 + BC_2)/2$         | 1    | 229.19** | 82.24**      | 0.678     | 39.38**     | 70.68**  | 163.35** | 7347.14**      | 38455.9**  | 81763.6**  | 193.94**     | 1981.0**  | 2292.3**  |  |
| Direct vs. indirect                 | 1    | 33.684   | 50.840**     | 1075.49** | 6.066*      | 4.389    | 447.24** | 10467.6**      | 5760.4**   | 495920.2** | 593.15**     | 1344.1**  | 15706.9*  |  |
| Error                               | 15   | 21.631   | 7.82         | 9.88      | 2.71        | 4.44     | 11.56    | 1078.5         | 1073.6     | 1535.72    | 29.61        | 27.14     | 43.85     |  |

\*and\*\* significant and high significant differences at the levels of probability (0.05 and 0.01, respectively).

#### **Estimation of maternal effects**

# a- By deviation of genetic component for cross breeding traits

Estimate the genetic components from data of cross breeding trials were calculated according to Dickerson (1992). Positive sign of  $h_n^2$ ,  $h_b^2$ , genetic advance% and heterosis Bp of Miskawi female minus male parent crosses recorded positive sign related to maternal force over berseem clover populations (Table 6-a).

Whereas, negative signs of genetic advance% and heterosis (Bp) of Giza-6 may be associated with environmental condition and variation between genotype.

#### Table 6-a. Maternal effects of total fresh weight plant<sup>-1</sup> over three crosses (Female parent minus male parent effects

| Genetic parameters                       | Giza-6 | Helaly | Hatour |
|--|--------|--------|--------|
| Narrow sense h <sup>2</sup> <sub>n</sub> | 0.42   | 0.66   | 0.60   |
| Broad sense h <sup>2</sup> <sub>b</sub>  | 0.33   | 0.33   | 0.06   |
| Genetic Adv%                             | -2.24  | 74.28  | 111.07 |
| Heterosis Bp                             | -35.47 | 84.59  | 48.26  |

Also, Table (6-b) showes positive sign of variation between female (Maternal) and male (Paternal) Miskawi effects among generations and crosses.

| Generations     | Giza-6 | Helaly | Hatour |
|-----------------|--------|--------|--------|
| Female parent   | 300.74 | 337.97 | 583.21 |
| F <sub>1</sub>  | 132.71 | 413.63 | 625.90 |
| F <sub>2</sub>  | 241.70 | 212.43 | 442.03 |
| F <sub>3</sub>  | 156.05 | 154.97 | 331.40 |
| BC <sub>1</sub> | 77.49  | 146.40 | 34.10  |

Table 6-b. Maternal effects of total freshweight plant<sup>-1</sup> over three crosses.

#### b- By Causal components of variance

expression significantly Trait was influenced by both maternal environmental and genetic components of variance. Environmental maternal effects ( $\sigma^2 Em$ ) were significant and substantial for both of total fresh and dry weight (g) plant<sup>-1</sup> (Table 7). Significant additive genetic variation was found for all traits under study, except for plant height and number of tillers of  $(\sigma^2_{Am})$  (Tables 7-a). Direct and maternal additive genetic variance  $(\sigma^2_{Ao})$  and  $(\sigma^2_{Am})$  were contributed to the expression of yield in all crosses (Giza-6, Helaly and Hatour) (Table 7-a). Total fresh and dry weight (g) plant<sup>-1</sup> were strongly influenced by all forms of genetic variation. However, the maternal additive genetic effects, including variance components, had stronger statistical support for number of tillers and plant height than direct genetic effects.

A comparison of rijAo and rijAm revealed fundamental similarities between the two matrices (Table 7-a). The genetic correlation of crossgenerations was nearly,  $r^2 = 0.91$  and  $r^2 = 0.98$ , for both of total fresh weight g and dry weight g in the three crosses (Table 7-b). Large negative correlations indicate that direct effects for plant height and number of tillers share an underlying genetic basis. The sign of the direct additive genetic correlations (rijAo) between-trait and the maternal additive genetic correlations (rijAm) between-trait was consistent (Table 7-a). This common pattern of relationship between traits for both forms of additive genetic variance suggests a similar underlying genetic basis that influences trait generations (rijA<sub>0</sub>, rijA<sub>m</sub>) compared with the correlation structure between traits and across generations (Räsänen & Kruuk, 2007). This contrast sing between direct  $(rijA_0)$  and maternal (rijA<sub>m</sub>) correlations indicated the presence of maternal force in traits of cross generation under study.

The force of maternal effects in berseem cultivars sustains the harmonious structure of population gene pool within each cultivar, enabling us to reduce isolation distance of foundation and breeder seed propagation in berseem fields.

Figure (2a,b and c) shows the plants of Fahl parent type in different successive cross generations (F<sub>1</sub> and BC, respectively), as well as Miskawi parent and the same cross generations (Figure  $2_{d,e \text{ and } f}$ ). The figure shows the tillers and branches developed by crossing in the first cut stage. The relative tillers number and plant vigor between female and male parents is observed. It is clear that recurrent selection for vigor individual plants is the best method to improve berseem clover populations in breeding program (Figure  $2_g$ ).

Such characteristic were observed in Figure 3 across each female parent, more than one tiller of Fahl female parent selected plants (Fig.3-1), whereas more branches and sub-branches per tillers of Miskawi female parent selected plants (Fig. 3-2).

| Characters         |                   | Giza              | -6             |                | Helaly            |                  |                |                | Hatour          |                    |                |                |
|--------------------|-------------------|-------------------|----------------|----------------|-------------------|------------------|----------------|----------------|-----------------|--------------------|----------------|----------------|
|                    | $\sigma^{2}_{A0}$ | $\sigma^2 A_m$    | $\sigma^2 E_0$ | $\sigma^2 E_m$ | $\sigma^2_{A0}$   | $\sigma^2 A_m$   | $\sigma^2 E_0$ | $\sigma^2 E_m$ | $\sigma^2_{A0}$ | $\sigma^2 A_m$     | $\sigma^2 E_0$ | $\sigma^2 E_m$ |
| Plant height<br>cm | 0.93<br>±0.94     | 5.41<br>±2.32     | 18.5           | 54.35          | 1.25<br>±1.11     | 1.95<br>±1.40    | 52.08          | 52.1           | 0.43<br>±0.66   | 2.82<br>±1.67      | 2.69           | 10.20          |
| No. tiller         | 0.15<br>±0.38     | 0.098<br>±0.31    | 2.00           | 1.50           | $1.05 \pm 1.01$   | 5.10*<br>±2.26   | 8.80           | 1.70           | 1.24<br>±1.11   | 2.64<br>±1.62      | 12.40          | 4.30           |
| Fresh yield g      | 410.5**<br>±20.26 | 472.3**<br>±21.73 | 4206*          | 5216*          | 826.3**<br>±28.74 | 1536**<br>±39.20 | 11109*         | 3888*          | 62.8**<br>±7.93 | 963.48**<br>±31.04 | 1488.3*        | 7512*          |
| Dry yield g        | 26.69**<br>±5.16  | 36.74**<br>±6.06  | 107.0*         | 131.8*         | 42.61**<br>±6.53  | 65.15**<br>±8.07 | 286.2*         | 101.3*         | 10.41*<br>±3.22 | 34.58**<br>±5.88   | 373.6*         | 144.3*         |

Table 7-aCausal components of variance for the univariate model of maternal inheritance that best describes each trait in<br/>over three crosses (female and male parent effects)  $\pm$  SE .

Whereas: the direct additive genetic variance ( $\sigma^2_{A0}$ ), the maternal additive genetic variance ( $\sigma^2 A_m$ ), the residual environmental component ( $\sigma^2 E_0$ ) and the maternal environmental ( $\sigma^2 E_m$ ).

Approximate standard errors are given in parentheses as well as the significance associated with the variance estimate. \*, P < 0.05; \*\*, P < 0.01.

| Table 7-b.         Direct (rijA <sub>0</sub> ) and maternal | (rijA <sub>m</sub> ) correlations between studied traits. |
|---|---|
|---|---|

| Giza-6            |                      |                      |                |                 |                      | Не                | elaly          |                 | Hatour               |                   |                |                    |
|-------------------|----------------------|----------------------|----------------|-----------------|----------------------|-------------------|----------------|-----------------|----------------------|-------------------|----------------|--------------------|
| Direct →          | Fresh<br>weight<br>g | Plant<br>hight<br>cm | No.<br>tillers | Dry<br>weight g | Fresh<br>weight<br>g | Plant<br>hight cm | No.<br>tillers | Dry<br>weight g | Fresh<br>weight<br>g | Plant<br>hight cm | No.<br>tillers | Dry<br>weight<br>g |
| Fresh<br>weight g |                      | -0.45                | 0.73**         | 0.98**          |                      | -0.40             | -0.04          | 0.96**          |                      | 0.03              | -0.48*         | 0.91**             |
| Plant hight<br>cm | 0.17                 |                      | 0.07           | -0.33           | 0.30                 |                   | -0.77**        | -0.36           | 0.85**               |                   | -0.46*         | -0.02              |
| No. tillers       | 0.77**               | 0.58*                |                | 0.82**          | 0.73**               | 0.79**            |                | -0.19           | 0.93**               | 0.73**            |                | -<br>0.64**        |
| Dry weight<br>g   | 0.97**               | 0.30                 | 0.75**         |                 | 0.99**               | 0.30              | 0.72**         |                 | 0.99**               | 0.90**            | 0.90**         |                    |

rijAo are above the diagonal and rijAm are below the diagonal







Figure 3. Tillers distribution among mono (Fahl) and multi-cut (Miskawi) crosses: 1) selected BC plants of Fahl female parent and 2) selected BC plants of Miskawi female parent.

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