Effect of Nitrogen Regimes on Combining Ability Variation in Oil and Protein Contents in Cottonseed (*Gossypium hirsutum* L.)

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**Abstract**: Eight parental cultivars and 56 hybrids of cotton (*Gossypium hirsutum*) were grown in normal and nitrogen-deficient conditions in the field to investigate the effect of nitrogen regime on the oil and protein contents and their combining ability. The mean oil and protein contents of the parental cultivars greatly varied with the nitrogen regime, which indicated their nitrogen sensitivity. The genetic variability of oil and protein contents was low in the same nitrogen regime, but was high in the comparison between the normal and nitrogen-deficient conditions. The nitrogen regime affected not only the oil and protein contents and ranking of parental cultivars, but also the combining ability. A normal nitrogen level was found to be more useful for selecting the additive type of gene action in breeding of cotton.

**Key words**: Genetic variability, *Gossypium hirsutum* L, Nitrogen regimes, Oil, Protein.

Increasing fiber production, producing high-quality fiber, breeding varieties ripening earlier, mechanical harvesting, and having improved resistance against diseases and pests are important objectives of cotton breeding these days (Christopher et al., 2003). Cottonseed is a major source of oil, and ranks second among the five major oilseed crops i.e., soybean, cotton, sunflower, peanut and rapeseed. Competition with other seed sources in the oil and the developing prospect of using cottonseed as a food, have increased the awareness of the potential importance of cottonseed to food reserves of the world.

In Pakistan, cottonseed (*Gossypium hirsutum* L.) is the major source of edible oil and has been reported to contribute 72% of the total edible-oil production (Khan et al., 1995), while the protein is fed to livestock and milch animals in the form of seed cake. Hence, cottonseed is an important source of providing nutrients. Since upland cotton is grown mainly for fiber production, breeders have conducted studies to exploit the potential of plant with a higher cottonseed yield and improved fiber characteristics. Thus due to a unidirectional breeding approach, increase in oil and protein contents has never been taken into consideration.

From the viewpoint of breeding, understanding genetic control of the desired traits like oil and protein contents of cottonseed is necessary along with factors influencing the magnitude of genetic variability associated with them. The supply of nitrogen throughout the growing season is one of the key environmental factors for a high yield, but nitrogen supply to the commercial cotton fields varies considerably due to various fertilization rates, soils nutrient composition and variation in rainfall (Lammerink and Morice, 1970; Peter, 2000).

The effects of stress environment and genotype on cottonseed yield, seed quality and fiber properties in cotton have been reported (Oosterhuis, 1999; Rahman, 2004; Rahman et al., 2005). Information is also available on the effect of nitrogen on the oil and protein contents, which indicated that nitrogen fertilization tends to increase the protein content of seed at the expense of oil (Scott et al., 1973; Nuttal et al., 1989; Boquet et al., 1993). However, there is no available information on the combining ability for oil and protein contents in different nitrogen regimes. This is partly due to a lack of understanding of the genetics of the traits involved. The objective of the present study was to determine the influence of different nitrogen regimes on inheritance of oil and protein contents in cotton (*Gossypium hirsutum* L.). Such information would help in planning comprehensive breeding programme aimed to improve oil and protein content in *G. hirsutum* seeds.

**Materials and Methods**

1. **Parental Material**
   The plant material consisted of four indigenous cultivars, MNH-93, MS-84, AC-134, and NIAB-78 and four exotics namely COKER-201, COKER-3113, Hg-H.N-134, and HAR-M-32-7-4. These cultivars were maintained by self-fertilization for several generations at the Department of Plant Breeding & Genetics, University of Agriculture Faisalabad. These genotypes differed in oil and protein contents and were
comparable in maturity duration (Khan et al., 1995).

Seeds of the parental cultivars were grown from mid November 2002 to mid March 2003, in 30 cm ×30 cm earthen pots (containing: a mixture of equivalent volume of sand, soil and farmyard manure) in a greenhouse. Day and night temperatures were maintained at 30°C and 25°C, respectively, using steam as well as gas heaters. The plants were exposed to natural sunlight and supplemented with artificial light, to provide a photoperiod of 16 hours. Seedlings were thinned to one plant per pot two weeks after planting. During growth and development, 0.25 g urea (46%N) was supplied to each pot every two weeks and plants were watered daily. The genotypes were crossed in a diallel fashion including reciprocal crosses to obtain seed of 56 hybrids. The parental cultivars were maintained by self-pollination.

2. Raising of experimental material

The experimental plant materials were grown in the field of Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Pakistan. The University Campus is situated 31-26° N latitude, 73°-06' E, at an elevation of 184.5 meters from the sea level, and in the center of the mixed cropping zone of the Punjab province. The soil samples were taken randomly from the field and analyzed for N-P-K contents. The experimental fields had a loam soil texture with a soil pH of 8.5, contained 0.93% organic matter, 27% saturation, 29.6 ppm available phosphorous and 139 ppm potassium. In an attempt to deplete the residual nitrogen, wheat was sown without fertilization, mowed 100 days later and all residual material was removed. The experimental area was divided into two parts; one was fertilized with 67 kg per hectare of nitrogen at the time of sowing. The other was supplied with 320 kg of nitrogen per hectare in three splits i.e., 67 kg at sowing and 1st irrigation, and 186 kg at the time of flowering as recommended by Institute of Soil and Environmental Sciences. Thus, the plants grown with 67 kg nitrogen were under “stress” as compared with the plants in the plot with a normal production package. The seeds of the F1 along with their parents were field planted in both the plots during the crop season 2003-04, following completely randomized block design with three replications in a single-row plot having distance between the rows was 75 cm and between plants 30 cm.

3. Estimation of Oil and Protein content

The cottonseed samples matured under nitrogen-stressed and “normal” conditions were collected separately. In this study, bolls were sampled only from the first two positions on the sympodial branches and randomly from all the branches to eliminate positional effect on seed physical traits. The healthy bolls were randomly excised from each plot carefully after the dew had evaporated i.e. between 10 and 11 am and put

| Source of variation | D.F | Mean Sum of square | Oil contents (%) | Protein contents (%) |
|---------------------|-----|--------------------|------------------|---------------------|
| Replications        | 2   | 0.06 NS            | 0.43**           |
| Nitrogen regimes    | 1   | 171.51**           | 67.08**          |
| Genotypes           | 63  | 24.24**            | 9.3**            |
| Parents             | 7   | 25.70**            | 25.71**          |
| Crosses             | 55  | 24.27**            | 7.18**           |
| Direct crosses      | 27  | 29.11**            | 8.56**           |
| Reciprocal crosses  | 27  | 19.88**            | 6.04**           |
| Direct vs. reciprocal (crosses) | 1 | 11.91** | 0.56** |
| Parents vs. crosses | 1   | 12.44**            | 14.65**          |
| Nitrogen regimes × genotypes | 63 | 5.11** | 2.04** |
| Nitrogen regimes × parents | 7 | 2.38** | 0.69** |
| Nitrogen regimes × crosses | 55 | 4.40** | 2.22** |
| Nitrogen regimes × direct crosses | 27 | 3.95** | 2.65** |
| Nitrogen regimes × reciprocal crosses | 27 | 4.34** | 1.86** |
| Parents vs. crosses × nitrogen regimes | 1 | 63.22** | 1.86** |
| Nitrogen regimes × direct vs. reciprocal | 1 | 15.48** | 0.12** |

** = Highly significant (P<0.01).
ns = non-significant (P>0.05).
in the Kraft paper bags. Clean and dry sample of the cottonseed were weighed and then ginned separately with a single roller electric gin in the Fibre laboratory. Oil contents were estimated by NMR (Nuclear Magnetic Resonance) and protein contents by Micro Kjeldhals method.

4. Statistical analysis

The data of oil and protein contents were subjected to ordinary analysis of variance with a factorial arrangement. All the effects were assumed fixed. Parental cultivars had been maintained by self-pollination (inbreeding coefficient, $F=1$). Method I, Model I described by Griffing (1956) was used for combining ability analysis.

Results

The values shown in Table 1 revealed that the protein and oil contents varied significantly with the genotype ($p<0.01$). Eight parental cultivars and 56 crosses also showed significant differences for both traits ($p<0.01$). The parents versus crosses data showed significant differences for both traits ($p<0.01$), which indicated that the mean performance of crosses deviated considerably from that of their parents, signifying the presence of hybrid vigour for the two traits. The data showed significant differences in direct versus reciprocal crosses for both traits ($p<0.01$), which indicated that the mean performance of direct crosses deviated considerably from that of their reciprocals, signifying the presence of cytoplasmic effects for the two desirable traits. The nitrogen level affected the performance of genotypes as indicated by the presence of significant interaction ($p<0.01$) between the genotypes and nitrogen regimes in both traits. This indicated that relative ranking among genotypes for both traits was not consistent across nitrogen levels. Parents x nitrogen regimes interaction also showed a significant difference ($p<0.01$), indicating substantial shift in relative ranking of parental cultivars for both traits over nitrogen regimes. Crosses x nitrogen regime interaction were found significant for both traits ($p<0.01$). Similarly variation due to direct and reciprocal crosses over nitrogen regimes was also found significant ($p<0.01$). Significant interaction among nitrogen regimes x parents versus crosses was also observed for both traits ($p<0.01$) which suggest that nitrogen regimes had significant effect on the hybrid vigour (Table 1). The significant interaction between nitrogen x direct versus reciprocal crosses indicated that nitrogen regimes had significant effect on cytoplasmic variations.

Combining ability for oil and protein contents was studied separately under nitrogen stress and non-stress regimes (Table 2). Substantial role of general combining ability, specific combining ability and reciprocal effects was evident for both traits and regimes (Table 2). Contribution of general combining ability was highest under normal nitrogen level while under nitrogen stress cytoplasmic differences were the main the contributors to the total genetic variability for both traits (Table 3). Relative contribution of general and specific combining ability decreased while variability due to cytoplasmic effects increased under nitrogen stress as compared to normal nitrogen level for oil contents (Table 3). For protein contents, relative contribution of specific combining ability and cytoplasmic genes increased under nitrogen stress as compared to normal regime (Table 3).

The effect of the nitrogen level on the phenotypic expression of parental cultivars for oil and protein contents was also evident (Table 4). The oil content of the parental cultivar varied with the nitrogen regime stress Cultivars AC-134, MS-84, COKER-3113, NIAB-78 showed increased oil contents under nitrogen stress while the parents MNH-93, COKER-201, Hg.H.N-134, and HAR-M-32-7-4 showed a decreasing trend under nitrogen stress (Table 4). The effect of nitrogen regime was also apparent on the general combining ability (GCA) expressed by the parental cultivars. The performance of parental cultivars regarding GCA for both traits was not consistent over nitrogen regimes in most cases and the relative ranking of the parental cultivars was altered (Table 4). COKER-201 was the most responsive to nitrogen fertilization and showed the highest reduction for oil contents under nitrogen stress. This cultivar showed the highest GCA for oil contents under a normal level of nitrogen. However,

| Sources of variation | D.F | Mean sum of square |
|----------------------|-----|--------------------|
|                      |     | Oil contents (%)   | Protein contents (%) |
|                      |     | Normal | Stress | Normal | Stress |
| GCA                  | 7   | 3.74** | 2.35** | 15.00** | 6.47** |
| SCA                  | 28  | 6.74** | 2.59** | 0.32**  | 1.30** |
| RE                   | 28  | 7.06** | 4.11** | 0.08**  | 1.48** |
| Error                | 126 | 0.08   | 0.08   | 0.003   | 0.01   |

** = Highly significant ($P<0.01$).
under nitrogen stress, H.g.H.N-134 showed the highest positive GCA for oil contents.

In all cultivars, protein content decreased under nitrogen stress. H.g.H.N-134 had the highest mean for protein contents under both regimes as compared to other cultivars. It also showed significant highest positive GCA effect for this trait (Table 4). The study on the association between performance of parents with that of the GCA (Table 4) revealed high correlations between GCA and performance for both traits under normal conditions.

### Discussion

Understanding of the type of genetic variability over and within environments is not only helpful in organizing a comprehensive breeding programme, it also provides a basis for determining trait(s) most likely to be improved under specific environmental conditions.

The magnitude of genetic variability for all traits over environments was low, but the genetic component within the environment was high. This is concurrent with the finding of Azhar and Ajmal (1999, 2000) who reported substantial genetic variability for both traits.

### Table 3

Estimates of components of variance and their percentages due to general combining ability (GCA), specific combining ability (SCA) and reciprocal effects (RE) for oil and protein contents over nitrogen regimes in upland cotton.

| Sources of variation | Oil contents (%) | Protein contents (%) |
|----------------------|-----------------|---------------------|
|                      | Normal          | Stress              | Normal          | Stress              |
| GCA                  | 3.74*           | 1.41                | 0.92            | 0.32                |
|                      | 49.87**         | 40.06               | 80.70           | 17.78               |
| SCA                  | −0.18           | −0.01               | 0.18            | 0.73                |
|                      | 2.40            | 0.28                | 15.79           | 40.56               |
| RE                   | 3.50            | 2.02                | 0.04            | 0.74                |
|                      | 46.67           | 57.39               | 3.51            | 41.11               |
| Error                | 0.08            | 0.08                | 0.003           | 0.01                |
|                      | 1.07            | 2.27                | 0.26            | 0.56                |
| Total                | 7.50            | 3.52                | 1.14            | 1.80                |
|                      | 100             | 100                 | 100             | 100                 |

*= Upper value denotes variance estimates.
**= Lower value denotes variance component in percentage.

### Table 4

Mean phenotypic expression (MP) of parents and general combining ability effects (GCA) for oil and protein contents in upland cotton under nitrogen stress and non stress regimes in the field.

| Genotype     | Oil contents (%) | Protein contents (%) |
|--------------|------------------|----------------------|
|              | Normal          | Stress              | Normal          | Stress              |
|              | MP | GCA          | MP | GCA          | MP | GCA          | MP | GCA          |
| MNH-93       | 25.64 | 0.22 | 24.21 | 0.45 | 15.11 | −1.18 | 14.10 | −1.00 |
| AG-134       | 22.30 | −0.47 | 3.13 | −0.50 | 18.10 | −0.18 | 17.34 | −0.35 |
| MS-84        | 23.54 | −0.35 | 24.38 | −0.12 | 18.02 | −0.08 | 17.19 | 0.10 |
| COKER-201    | 24.73 | 0.54 | 21.49 | 0.17 | 15.03 | −1.32 | 14.17 | −0.70 |
| COKER-3113   | 25.28 | −0.56 | 25.49 | −0.53 | 19.82 | 0.72 | 18.38 | 0.58 |
| NIAB-78      | 23.81 | −0.36 | 24.99 | 0.18 | 19.46 | 0.66 | 17.42 | 0.29 |
| Hg.H.N-134   | 25.34 | 0.52 | 24.42 | 0.47 | 21.78 | 1.55 | 19.45 | 0.87 |
| HARM-M32-74  | 24.09 | 0.46 | 23.36 | −0.12 | 17.72 | −0.17 | 17.36 | 0.21 |

*CD (g-g) = Critical difference (P<0.05).
**= Highly significant (P<0.01).
ns = non-significant (P>0.05).
The results revealed significance of both general, specific combining ability and cytoplasmic variations in the inheritance of oil & protein contents. This finding is in agreement with those reported by Dani and Kohel (1989), Wang and Li (1991), and Khan et al. (1997) who showed that oil and protein contents were controlled by both additive and non-additive gene effects. Both oil and protein contents exhibited a larger proportion of non-additive genetic variation in the presence of nitrogen stress while an additive type of gene action was found under a normal nitrogen level. Nitrogen-stress conditions were more favorable for the expression of variability due to cytoplasmic effects. Wu et al. (1995) also revealed the importance of cytoplasmic genes in the inheritance of oil contents. This would have implication on the selection of the environment and the breeding procedure to be adopted for improvement of oil and protein contents. The findings of the present study, although based on selected cultivars, suggested that segregating generations grown under a normal nitrogen level would show a better response to the selection because the normal nitrogen level promoted the additive type of genetic variability. The high correlation among performance and additive type of genetic variability (GCA effects) under a normal nitrogen level suggested that the ability to transmit desirable characters to progeny can be predicted even from the phenotypic performance of a plant. Thus, parents showing a higher mean performance for oil and protein contents may be utilized to establish superior progenies under normal nitrogen level. Intermating and reciprocal recurrent selection between good general combiner would be useful breeding procedure for accumulating favorable genes for oil contents and protein contents under nitrogen stress regime. The cultivars Hg.H.N-134 and NIAB-78, were good general combiners for oil and protein contents and could serve as the base population for intermating and reciprocal recurrent selection and may produce superior recombinants in segregating progenies.

Conclusion

In conclusion, both oil and protein contents of cottonseed are influenced by the nitrogen regimes and genetic variability is likely to be increased under a normal nitrogen level.

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