CHAPTER 5

DISCUSSION
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Sulphur requirement and metabolism in plants is closely related to N nutrition, whereas N metabolism is also strongly affected by the sulphur status of the plant (Duke and Reisenauer, 1986). Sulphur requirement of crops vary greatly depending upon the crop species, yield levels, the sulphate-S concentration in the soil solution, the levels of fertilizer N, P and K applied to crops, etc. In general, S demand of Cruciferae and Liliaceae is highest and lowest of small grains, while Leguminosae range in between. According to Walker and Booth (1992), the rapeseed crop removes between 20 and 30 kg S ha\(^{-1}\), while cereals remove about 10 to 15 kg S ha\(^{-1}\).

The experimental findings presented in the preceding chapter provide a detailed account of the physiological and biochemical bases of performance of groundnut and soybean cultivars, assessed in terms of growth, development, yield attributes and seed as well as oil yield, as influenced by S and N nutrition.

5.1 Growth Attributes

Growth is defined as a process of expansion or an increase in size, where size may be described by dimension of weight or length. Development, on the other hand, describes the passage of a plant through its life cycle. Growth and development are interdependent processes and both are influenced by several endogenous and exogenous factors, the vital among them is nutrient availability. It is imperative that the processes involved in determining the yield of groundnut and soybean must be considered in depth before analysing the component, which may be important in determining the yield. Seed yield is a function of growth and development attained, production of biomass and its partitioning into the component, which constitute yield. In this section various growth attributes like total dry matter and its partitioning in various plant parts, leaf area index, leaf area duration and crop growth rate will be discussed.

A perusal of the response exhibited by cultivars in present study reveals that groundnut and soybean cultivars differed significantly in respect of growth attributes at almost all the growth stages. The groundnut cultivar, Kaushal produced 66.47 per cent more biomass than Amber and soybean cultivar, PK-1024 produced 5.07 per cent more biomass than PK-416. Since the ability of the plant to produce dry matter depends upon the size, efficiency and duration of photosynthetic organs, which is measured in terms of leaf area index (LAI), and leaf area duration (LAD). Hence on examining these
parameters, it was found that groundnut cultivar Kaushal had higher LAI and LAD, when compared with the cultivar Amber while soybean PK-1024 had higher LAI and LAD, when compared with the cultivar PK-416.

It is interesting to note that decline in LAI coincided with the pod development phase. The increase in pod dry weight and total dry weight, in spite of the rapidly declining leaf area, could be attributed to both the ability of the pods to photosynthesize and translocate the dry matter from vegetative parts viz., stem and leaves to meet the assimilate demands of developing pods and seeds. The studies of Allen and Morgan (1972, 1975), Tayo and Morgan (1975, 1979) and Thurling (1974a) on rapeseed mustard indicated the importance of pods as photosynthetic organs. It is evident from these observations that the sink demand for assimilates in soybean is met with the translocation of photosynthates from leaves, stem and also by the pod photosynthesis. The slight decline in total dry matter accumulation at harvest could be attributed either to sampling error or to enhanced respiratory losses during reproductive phase when temperatures are usually high. This was also reported in wheat (Abdin et al., 1996).

Groundnut and soybean had accumulated more biomass, and maintained higher leaf area index and leaf area duration with the application of sulfur along with nitrogen compared to the application of nitrogen alone, achieving the highest value when sulfur was applied at the rate of 20kg/ha along with nitrogen 40kg/ha in groundnut and 40kgS/ha along with 40kgN/ha in soybean. The high responses of these crops to the combined application of S and N may be due to the availability and supply of these nutrients to the crop during the entire growth period including the most crucial reproductive phase. Since both these nutrients are involved in the biosynthesis of proteins and many other important biomolecules, hence, a balanced application of S and N enhances their use efficiency in crop plants. The strong interaction between sulfur and nitrogen has been established in several studies in terms of dry matter and yield in several crops (Dev and Sagger, 1974; Jahan et al., 1992; Lakkieni and Abrol, 1992; Singh and Beirathi, 1980; Sachadev and Deb, 1990; Zhao et al., 1993; MacGrath and Zhao, 1996; Ahmad et al., 1998, 1999; Ahmad and Abdin, 2000; Abdin et al. 2003a, 2003b). There are many reports showing that application of S along with N increased the nitrogen use efficiency (Stewart and Porter, 1969, Eppendorfer, 1971, Smith 1975), and increased nitrogen level in plants enhanced the leaf number and leaf size leading to the increase in leaf area index (Murata and Matisushima, 1975; Hageman and Below, 1990). Further, better nitrogen status of leaf results into delayed senescence in the higher leaf.
area duration as observed in our study. Adequate N and S supply resulted in the better utilization of carbohydrates to form more protoplasm. The cells produced under such conditions tend to be large with thin walls (Black, 1967), which may cause increase in leaf area. The higher leaf area indicates better leaf area expansion. This leaf expansion helps in the subsequent interception and efficient utilization of the solar radiations, resulting in better accumulation and distribution of dry matter in leaves and shoots. The leaf area duration, the products of leaf area index and time, is also affected by change in the leaf area index with time and, S and N application.

5.2 Biochemical Attributes

Nitrate reductase and ATP sulphurylase activities differed in both the crops. The soluble protein content and chlorophyll content of the two crops, however, were almost equal.

Nitrogen assimilation pathway is closely linked to carbon assimilation. While the carbon fixation forms the carbon skeleton, the nitrogen assimilation provides reduced nitrogen, which is used in the synthesis of amino acids and protein. The latter is involved in mediating various steps of carbon cycle and thus, involved in the synthesis of organic assimilates and leaf organs. Nitrogen assimilation pathway is not only important for agricultural, but also for ecological and even economical point of view. The improvement of crop yield by enhancement of efficiency of nitrate reduction process is the ultimate goal of a variety of studies conducted on nitrate assimilation (Abrol et al., 1984; Abrol, 1990; Abdin et al., 1992, 1994; Ahmad et al., 1999). The nitrate reductase enzyme catalyses the first step of nitrate assimilation pathway (Beeveres and Hageman, 1969; Abrol et al., 1976), and hence, its product ultimately finds its destiny in protein which is associated with biochemical machine converting raw materials such as CO₂, H₂O and light into biomass. Application of S along with N significantly enhanced the NR activity compared to the application of N alone. The magnitude of enhancement was maximum, when S and N were applied at the rate of 20 and 40 kg/ha in groundnut and 40 and 40kg/ha in soybean cultivars respectively. The relationship between NR activity and the seed yield of the groundnut and soybean cultivars are presented in figure 32 & 34, as described by linear equation. A strong positive correlation of NR activity and seed yield (r = 0.975 in V₁ and r = 0.986 in V₂, in groundnut; r = 0.993 in V₁ and r = 0.966 in V₂, in soybean), was observed in groundnut and soybean cultivars. The correlation between NR activity and seed yield was also observed in other crops viz., maize (Deckard et al., 1973; Balaṣubramainium, 1977), Wheat (Croy and Hageman, 1970;
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Eilrich and Hageman, 1973; Reilley, 1976; Deckard et al., 1977; Abrol and Nair, 1978
and Nair and Abrol, 1982), and rapeseed -mustard (Ahmad et al., 1999). High NR
activity in plants coincided with more absorption of nitrate which is greatly affected by
availability of sulphate in soil and ATP-sulphurylase activity. Clarkson et al. (1989)
observed a marked depression in the ability of cereal plants to take up nitrate and
ammonium, when plants were starved of sulphate. A positive role of sulphate in
regulating nitrate reductase was found by Pal et al. (1976) and Friedrich and Schrader
(1978). Filner (1978) reported that the assimilation pathways of the S and N have been
considered functionally convergent pathways. The role of nitrogen in the regulation of
sulphate assimilation at the ATP-Sulphurylase step was observed by Smith (1975). The
work of Brunold and Suter (1984) on tobacco cell cultures demonstrated that, when
sulfur limited growth, the induction of NR by nitrate was proportional to the initial
sulphate concentration and, when nitrate limited growth, the depression of ATP-
sulphurylase was proportional to the initial nitrate concentration, and the
interrelationship of the regulation of NO₃⁻ and SO₄²⁻ assimilation was an effective
mechanism to co-ordinate and meet the requirements of net protein synthesis (Reuveny
et al., 1980). Similarly Barney and Bush (1985), while working on tobacco at the whole
plant level, reported that +N-S treated plant had very low NR activity because of the
lack of S, when plant were transferred from +N-S to -N+S. NR activity remained very
low because of the supply of the limited N in the plant, and it might have prevented
SO₄²⁻ translocation from roots to the shoots. Clarckson et al. (1989), while working on
barley plants, demonstrated that at the whole plant level the apparent matching of supply
to demand is accompanied by an apparent linkage of NO₃⁻ and SO₄²⁻ uptake. The
synthesis of cysteine as a result of the incorporation of sulphide moiety into O-
acetylserine appears to be meeting point between N- and S- metabolism. Naturally
occurring thiol compound viz., cysteine and glutathione were shown to influence nitrate
reductase activity in wheat and Brassica Sp. (Lakkinini and Abrol, 1992). These studies
are in accordance with our observation, where a positive relationship between the
activity of these two enzymes in groundnut and soybean cultivars was observed.
Application of S and N in combination increased the activity observed with the
application of 20 S and 40 N kg/ha in groundnut and, 40 S and 40 N kg/ha in soybean
cultivars. The increased in seed yield however can not be solely attributed to NR activity,
but also to ATP-sulphurylase activity as both N- and S-assimilation pathways are
interdependent. We have observed the same in this study. Figure 33 & 35 showed a
Fig. 32: Relationship between seasonal nitrate reductase activity of leaves and seed yield of soybean cultivars at different combination of S and N

\[ Y = -34.44 + 7.61x \ (r=0.993**) \]

\[ Y = -20.56 + 6.77x \ (r=0.966**) \]
Fig. 33: Relationship between seasonal ATP-sulphurylase activity of leaves and seed yield of soybean cultivars at different combination of S and N.

For Glycine max cv. PK-416:

\[ Y = -6.12 + 1.22x \quad (r=0.948^{**}) \]

For Glycine max cv. PK-1024:

\[ Y = -5.09 + 1.25x \quad (r=0.956^{**}) \]
Fig. 34: Relationship between seasonal nitrate reductase activity of leaves and seed yield of groundnut cultivars at different combination of S and N

\[ Y = -43.47 + 6.53x \ (r=0.975^{**}) \]

\[ Y = -56.83 + 9.21x \ (r=0.986^{**}) \]

*Arachis hypogaeae* cv. Kaushal
**Arachis hypogaeae L. cv. Amber**

\[ Y = -1.83 + 1.62x \ (r=0.937^{**}) \]

**Arachis hypogaeae L. cv. Kaushal**

\[ Y = -1.69 + 2.31x \ (r=0.918^{**}) \]

Fig. 35: Relationship between seasonal ATP-sulphurylase activity of leaves and seed yield of groundnut cultivars at different combination of S and N.
strong positive correlation between the seed yield and ATP-sulphurylase activity as described by linear equation. Ahmad et al. (1999) reported the correlation between ATP-sulphurylase activity and seed yield in rapeseed-mustard. ATP-sulphurylase catalyzes the first step of S-assimilation pathway and, therefore, regulates the inflow of S like NR regulating the inflow of reduced N in plants. Increased NR and ATP-sulphurylase activities with the combined and split application of S and N to the crops were an indicator to increased metabolic activity related to S and N-assimilation.

The chlorophyll and leaf soluble protein content were increased significantly with the application of S and N, compared to the application of nitrogen alone. The inter-relationship between both attributes were also reported by Evans (1983), Makino et al. (1984), Lawlar et al. (1987a, b), Lawlar et al. (1989) and Sinclair and Horie (1989).

5.3 Biological Nitrogen Fixation

In pot experiments the treatments without S application were found to cause the shortage of S during early growing period in different legume crops. The plant growth was also reduced later in the growing season also. The yield response to optimum S application, however, differed among the crop species, being lower in Medicago sativa and Pisum sativum as compared to Trifolium pratense and Vicia faba, suggesting that legumes differ in their S demand (Scherer and Lange, 1996). Gupta and McLeod (1984) found that S supply only increased the S concentration of the plants without enhancing the yield. Although these studies show a link between S supply of legumes and N₂ fixation, only a few experiments have been conducted to investigate the influence of S nutrition on this process. Shock et al. (1984) have demonstrated that applied S increased the percentage of symbiotically derived nitrogen in subclover. With S-deficiency, amino acids and other N forms accumulate due to the impaired protein synthesis. This could be due to the feedback repression of nitrogen fixation (Janssen and Vitos, 1974). Recently, Scherer and Lange (1996) found the higher N accumulation with optimum S supply Lange (1998) suggested that S affects leguminous species through its effect upon nitrogen-fixation by Rhizobium species. According to these results, biological N₂ fixation, nodulation, PEP carboxylase activity and the protein concentration of legumes were reduced with severe S-deficiency, while moderate S-deficiency diminished the nodule formation and nitrogenase activity.

As compared to subterranean clover supplied with S, nodulation was markedly decreased in S-deficient clover. It is attributed to the decline in the requirement for N
with reduced S supply (Anderson and Spencer, 1950). However, the observed increase in the number of nodules by S fertilization of legumes was not the result of increased nodulation per unit length of roots, but due to the better root growth (Gilbert and Robson, 1984; Scherer and Langes, 1996).

Circumstantial evidence indicate that S-deficiency greatly diminishes carbon-fixation of *Medicago sativa* (Mertz and Matsumoto, 1956), which is assumed to be caused by the reductions in synthesis of key enzymes of the carbon metabolism as a result of reductions in the pools of the free S-containing amino acids, cysteine and methionine (DeBoer and Duke, 1982). Lange and Scherer (1996) investigated the effect of S nutrition on the activity of key enzymes of the C-and N- metabolism of *Vicia faba* and *Pisum sativum*. Their results show that with both the legumes, the activity of enzymes (g\(^{-1}\) fresh weight of nodules) significantly decreased under S deficiency conditions. However, the influence of S-supply on the activity of malate dehydrogenase and PEP-carboxylase was more pronounced as compared to glutamate-synthase.

In our investigation, combined and split application of S and N in the groundnut cultivars resulted in the significant increase of the number of nodules per plant and nodules weight and nitrogenase activity.

### 5.4 Distribution of Sulphur and Nitrogen in Various Plant Parts

Nutrient concentration in various plant parts at various crop growth stages indicates their relative requirement, total uptake and distribution. Plants generally absorb sulfur as SO\(_4^{2-}\) ion, which is translocated in an upward direction. The mobility of sulphur is low. This behavior, which is unlike that of N, can be useful in distinguishing between S and N deficiencies. The total sulfur required by different crops depends on the plant species and on the yield levels or the production of total dry matter. Crops with a high production of dry matter such as sugarcane and maize have a high demand for S. A high sulphur requirement is also characteristic of protein-rich crops, crucifers and Brassicas. The sulfur requirement of rapeseed-mustard is nearly three times that of cereals (Kanwar and Mudahar, 1986). Generally, the S concentration of most of the plants is between 0.1 per cent to 0.3 per cent. However, as high as 2.0 per cent S in mustard leaves has also been reported, whereas roots invariably have lower concentration of S (Kanwar and Mudahar, 1986).

Sulphur and nitrogen concentration in various plant parts at different growth stages of groundnut and soybean cultivars are discussed in the subsequent section.
Efforts are not only made to explain the variation existing in S and N concentrations in the groundnut and soybean cultivars, but also to analyze the effect of S and N interaction on N and S accumulation and N and S-harvest in these crops.

5.5 Nitrogen Concentration in Various Plant Parts, Total Plant Nitrogen and N-Harvest Index

Leaf nitrogen concentration was highest during early vegetative growth, which then exhibited a declining trend. This decline coincided with bolting and continued further in both the crops. The concentration of nitrogen in stem was less than that of leaves. S and N fertilization in various combination considerably increased the leaf and stem nitrogen concentration in both the crops compared to the application of N alone, thus indicating a strong interaction between S and N metabolism. Best results were obtained with the application of S and N at the rate of 20 and 40 kg/ha in groundnut and 40 and 40 kg/ha in soybean, respectively.

Sulphur application along with N also increased the pod nitrogen concentration in both the crops when compared to N alone. An increase in nitrogen concentration in pod with the various combinations of S and N indicates a role for sulfur in improving the efficiency of nitrogen metabolism. At harvest, the nitrogen concentration was highest in seed when compared to the other plant parts, suggesting the translocation of N from vegetative parts into the seeds. Sulphur and nitrogen fertilization in various combinations significantly improved the seed nitrogen concentration, attributing a role of sulphur in nitrogen transport into the seeds, achieving maximum values when S and N were applied at the rate of 20 and 40 kg/ha in groundnut and 40 and 40 kg/ha in soybean.

In spite of the same values of seed nitrogen, the two crops differed in terms of total nitrogen and nitrogen harvest index. Increase in the total plant nitrogen was observed with the application of S and N when compared to the application of nitrogen alone.

5.6 Sulphur Concentration in Various Plant Parts, Total Plant Sulphur and S-Harvest Index

Leaves and stem of the groundnut and soybean crop exhibited maximum sulfur concentration during the peak vegetative phase. The enhancement of sulfur concentration in the leaves and stem with sulfur and nitrogen fertilization is optimum with 20 kg S/ha and 40 kg N/ha in groundnut and, 40 kg S/ha and 40 kg N/ha in soybean indicating thereby a strong interaction between S and N metabolism.
An increase in the sulphur concentration in pods was evident right from the initiation of pods to maturity. Pod appeared to be the strong sink drawing sulphur from all plant parts. S and N application in different combination enhanced pod sulfur concentration compared to the application of nitrogen alone. Sulphur concentration in the pod decreased, while increased in seeds with age, suggesting translocation of sulfur from pod to the seed. High sulfur concentration in pod and seed was observed with the application of S and N at the rate of 20 and 40kg/ha in groundnut and 40 and 40kg/ha in soybean, respectively.

Kastori and Jocic (1995) observed a positive correlation between S and N of seeds in wheat. Many other studies also indicate synergistic effect of combined application of S and N on the uptake and assimilation of these nutrients (Aulakh et al., 1977,1980; Singh et al., 1980; Janzen and Battany, 1984; Biswas et al., 1995; Ahmad et al., 1998).

5.7 Yield Attributes
5.7.1 Yield Components
The growth attributes are primary requirement for the development of yield components viz. number of pods per plant, number of seeds per pod and 100 seed weight. Strong positive correlation between yield components and seed yield was observed by several investigators in various crops (Bahl and Jain, 1977; Salimath and Bahl, 1986). The yield components on the other hand are influenced by several factors such as genotypes, nutrients and environmental conditions.

The number of pod per plant and 100 seeds weight were enhanced with the application of N and S at the rate of 20 and 40kg/ha in groundnut and 40 and 40kg/ha in soybean, respectively. As discussed earlier, the application of S and N at this level increased the leaf area index, leaf area duration, crop growth rate, chlorophyll content, soluble protein concentration, enzyme activities, assimilate formation and its translocation from stem and leaves to the developing pods. Hence, crop supplied with balanced dose of S and N would naturally have the optimum number of pods per plant. The balanced dose of S and N would also optimize their size and length due to the availability of high amount of photoassimilates. Besides, increased supply of photosynthates to pods would also provide an opportunity for seed to grow upto their full size with obvious increase in 100 seeds weight as observed in our study. Favourable effects of S and N on these traits were also reported by several investigators (Zhao et al.,
simultaneous uptake of S and N increased pod abortion.

5.7.2 Seed Yield, Biological Yield and Harvest Index

Seed yield is a compound effect of yield components viz., number of pods per plant, number of seeds per pod and 100-seed weight, whereas, biological yield is an expression of overall growth of the plant. The difference in seed yields may thus, arise mainly as a result of differences in the yield components (Thurling, 1974b; Mehrotra et al., 1976).

The capacity of a plant to produced economic yield depends not only on the size of photosynthetic system, its efficiency and length of the time for which it is active, but also on the translocation of dry matter into economic sink. The final buildup is the cumulative function of yield components. Harvest index is an important trait in determining yield and represents increased physiological capacity to mobilized photosynthates and their translocation into organs of economic value.

Seed yield, biological yield and harvest index of both the crops were positively influenced by the application of S and N in various combinations, when compared with nitrogen alone. The magnitude of response measured in terms of seed yield, biological yield and harvest index of groundnut and soybean crops to the application of 20kg S/ha alongwith 40kg N/ha and 40kg S/ha alongwith 40kg N/ha was higher when compared with other combination of S and N. It is obvious, as being important plant nutrients, S and N availability in balanced doses could naturally boost crop growth, development and yield as observed in our study. Aulakh et al. (1977) also reported increase in the yield of mustard varieties (by 155-180%), when 20kg S/ha was applied alongwith N fertilizer. Zhao et al. (1993) reported that N application increased the seed yield through enhanced pod formation, while S application reduced pod abortion. The improvement in growth and yield attributes by S and N application finally led to the higher biological yield and enhanced seed yield. Application of S and N resulted in proportionally higher increase in the seed yield compared to the biological yield, which ultimately led to the higher harvest index. Our results are in uniformity with several earlier reports (Vass et al., 1977; Kostov et al., 1988; Naik, 1989; Yadav et al., 1990; Ahmad et al., 1998). A positive correlation between harvest index and economic yield was also observed in Brassica species earlier (Thurling, 1974a; Varshney and Singh, 1982).