Chapter — 5

Summary and Conclusions
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Legumes are important crops. In India, a variety of legumes are grown under a wide range of agroclimatic conditions and contribute 25 percent of the global legume production. Legumes are inseparable ingredients of vegetarian diet and one of the cheapest source of dietary protein in Indian subcontinent. India has largest area of cultivation of legumes in the world but average production is low and production is not sufficient, to meet per capita requirement. The daily per capita availability of legumes has decreased from 69 g in 1961 to 37 g in 2004.

Chickpea (Cicer arietinum L.) is one the most important legume. The crop is of great significance in India particularly for meeting the protein demand of the vegetarian population as well as for restoring soil fertility. It ranks second in area and third in production among the pulses in world. India is a leading country with the highest area among the 34 chickpea growing countries under diverse geographical conditions. India is a premier chickpea growing country in the world and ranks first in area (8.69 million hectare) and production (6.97 million tonnes) (Ali and Kumar, 2000). Chickpea is a very good source of carbohydrates and proteins, which together constitute about 80% of total seed, dry weight. Chickpea is consumed as dry pulse after cooking, germinating, soaking or fermenting or as a green vegetable with the former being most common way of consumption. Some snacks prepared are from the flour of Dhal. Various sweet and spicy dishes are made from split grains. Green leaves are used as vegetable. In advanced countries, the grains are sold in market in dry or canned conditions for common use in soups, vegetable combination or as salad. It is also a good source of livestock feed. Production and yield of this crop in India is merely 780 Kg/ha. The yield of this crop is stagnant. Net availability declined to from 22.5 to 10.8 g/capita/day during last five decades.

Various efforts were made for crop improvement in terms of practices, fertilizer application and genetic improvement, but the yield of chickpea remains low; the reasons for this are poor flowering and pod setting in some and in other cases heavy abscission of flowers, immature pods and poor pod filling, leaf senescence at pod setting, time due to low seed index. Some abiotic stresses like, terminal drought, high temperature during reproductive stage, cold sensitivity during the vegetative and flowering stages and salinity
throughout the crop period inflict major yield losses and instability in production (Ali and Kumar, 2003). Various agronomical, phonological, morphological and physiological factors affect the yield components. Integrated efforts are required to achieve potential yield of chickpea through multidisciplinary approach involving physiology, agronomy, biochemistry, breeding and molecular biology to manage various abiotic stresses. However, no significant breakthrough has been achieved till date due to poor understanding of key physiological and biochemical processes regulating yield components, complex response of chickpea to environment (Genotype × Environment interaction), genetic components and their stability towards diverse climate.

Seed is the most basic and cheapest yet most crucial input to realize high yields and increased productivity. Seed serves as basic propagule, the starting point for many crops. The great majority of many agriculturally important plants are propagated from seed, thus for better production and maximum yield; seed also plays a paramount role.

The beneficial role of plant growth regulators and phenols, applied though either foliar sprays or seed soaking treatments, is well recognized in increasing the germination efficiency, growth performance and also yield components in some crops. Presoaking seed treatments is beneficial for good start of any crop (plant), it also enhances plant growth and development and it gives also better results in case of harvest and yield. Increase in yield due to growth regulators could be attributed due to increased morphological, physiological and growth characters coupled with yield attributes. Application of growth regulators might increase assimilation rate and growth rate of plants, which is evident from increased leaf area, chlorophyll content and soluble protein (Prakash et al. 2003). The beneficial effects of plant growth regulators (PGRs) and phenols have been reported for their ubiquitous role in controlling many facet of life cycle. They may bring about favorable shift in various physio-bio-chemical aspects which offer an immense practical application of bioregulants in agriculture. Plant growth regulators are known to regulate flowering, fruit setting and fruit development. Production and yield are also increased with the help of PGRs and phenols.

Germination performance under (laboratory conditions as well as field conditions) plays important role in crop yield. Seedling vigour index and quality index are important factors for better of germination. Changes in metabolites and enzymes during germination of
seedlings raised from treated and untreated (Control) seeds is important study for mobilization of metabolites to cotyledons to embryonal axis during germination and one can say whether field stand of seedlings is good or not.

Presowing presoaking treatments with PGRs and phenols are required to be correctly applied both in terms of correct dosage rate and uniform application, therefore proper selection of PGRs and phenols, proper time for soaking and volume for each type of cultivar is necessary for their regulatory effects.

Foliar application of PGRs and phenols to the crop at proper stage is one of the important factor for subsequent yield of any crop. Thus appropriate time/stage for foliar application during crop growth is key factor. Foliar application showed positive effect on seed maturation and grain filling. Right time of harvest of seed is also important; and sufficient accumulation of metabolites, maximum dry weight is also considered. Seeds of chickpea accumulate major protein content. The study of its fractions and eletrophoretic banding pattern is rewarding.

Crop plants grow by photosynthesis. Thus, plant productivity in terms of primary production of biomass is simply a measure of the total photosynthesis of the plants less respiration, which has occurred during its growth. Agriculturists are, however, concerned more with economic yield than with total biomass. The proportion of total biomass production, which is invested into harvested parts of the plant is termed the harvest index (HI). (Sharma and Ghildiyal, 2005).

The improvement in photosynthetic performance seems to have considerable scope of improving crop yield. Plant productivity is to a large extent determined by efficiency of photosynthesis under given environmental conditions and available resources (Kalpana et al. 2003). Seed yield is related with photosynthetic rate, transpiration rate and stomatal conductance. So study of various photosynthetic parameters during growth and yield of crop is the critical point. In case of legumes, specially economic legumes, there is lack of this type of study. There is study for soil moisture, genetic variability, fertilizers or salinity. So this type of study specially for photosynthesis related to growth and development and yield, it can give ideas about source – sink relations, dry matter accumulation and accumulation of metabolites of seed during maturation, plant type and how can delay senescence and how can stop flower shedding.
End – product inhibition of photosynthesis as well as the effect of photosynthesis on translocation of organic constituents are key factors affecting the source – sink relationships in crop plants.

Hence keeping these views in mind, the work has been carried out on four important cultivars of *Cicer arietinum* L. cv. ICCC 4, Dahod yellow, Gujarat – 1 and Gujarat – 2 with the following objectives.

**Objectives:**

**Experiment – 1 STUDIES DURING GERMINATION (Chapter – 2)**

I. **To undertake studies on presoaked seeds of chickpea (*Cicer arietinum* L.) (Desi) during germination.**
   
   (i) Standardization of methods for presoaking treatments for soaking duration volume and concentration of PGRs and to evaluate the changes in enzyme activity and metabolites in PGRs presoaked germinating seeds.

   (ii) To test the performance of presoaked seeds during germination

**Experiment – 2 STUDIES ON GROWTH, DEVELOPMENT, YIELD AND PHOTOSYNTHESIS (Chapter – 3)**

II. **To understand the impact of seed soaking and foliar sprays with PGRs on chickpea varieties under field conditions**

   (i) Meteorological data during growth and development

   (ii) Growth and development, flowering and fruiting, yield and harvest under field conditions under the effect of foliar application of PGRs and presoaking seed treatments

   (iii) To carry out studies of PGRs on different photosynthetic parameters under field conditions.

**Experiment – 3 STUDIES ON SEED MATURATION (Chapter – 4)**

III. **Morphological, physiological and biochemical studies on maturation and electrophoretic patterns of mature seeds**

   (i) To undertake studies on seeds during maturation of plants grown from presoaked seed treatments and foliar sprayed plants

   (ii) To evaluate qualitative electrophoretic patterns of proteins, of mature seeds.
EXPERIMENT - 1: STUDIES DURING GERMINATION (Chapter - 2)

The laboratory studies include seed soaking, leachate analysis, germination and metabolites and enzyme activities during germination. Standardization of volume and duration of soaking treatments with four cultivars of chickpea using DW as soaking solution/media. Effective volume and duration is very important for further presoaking with PGRs and phenols. Various PGRs in a wide range of concentrations were applied e.g. gibberellic acid (GA$_3$) (50, 100, 150 ppm), Kinetin (KIN), (10, 20, 30 ppm) Maleic hydrazide (MH) (100, 200, 300 ppm) and phenols like salicylic acid (SA) (10, 20, 30 ppm) and caffeic acid (CA) (25 ppm).

Soluble metabolites viz. Starch, Sugar, Protein, Phenol, Inorganic phosphate, RNA and DNA were analyzed during different hours of germination. These metabolites were also analyzed for maturation studies during different stages of maturation during seed and pod formation stage. The study of enzyme activities viz. Amylase, Invertase, Protease, Peroxidase, Enzyme Protein, Polyphenol oxidase, IAA Oxidase were carried out. Germination performance on root length, shoot length, percent germination, quality index and seedling vigour index were carried out. For acquaintance/knowledge of potentiality of new seed lot, the leachate analysis was also carried out.

- There is no difference in (presoaking treatments with DW) durations or volume every cultivar is different. The effective soaking volume (DW) and duration among all treatments is 1g/2ml for 5 hours. The effective concentration of PGRs and phenols is different for each cultivars, like wise, For ICCC 4 Gibberellic acid (GA$_3$) 150 ppm, Salicylic acid (SA) 20 ppm and Caffeic acid (CA) 25 ppm, for Dahod yellow Gibberellic acid (GA$_3$) 50 ppm, Salicylic acid (SA) 30 ppm and Caffeic acid (CA) 25 ppm, for Gujarat – 1 Gibberellic acid (GA$_3$) 100 ppm, Salicylic acid (SA) 30 ppm and Caffeic acid (CA) 25 ppm, for Gujarat – 2 Gibberellic acid (GA$_3$) 150 ppm, Salicylic acid (SA) 20 ppm and Caffeic acid (CA) 25 ppm.

- Germination performance of seedlings raised from presoaked seeds showed better seedling growth and also recorded higher seedling vigour index I and II and quality index as compared to control.

- The presoaked treatments of gibberellic acid and caffeic acid were better among as compared to other treatments in case of laboratory germination. Percent
germination did not show any variation with treatments because the seedlot is new

- Seedlings from presoaked seeds showed higher enzyme activity and free soluble metabolites in their axis during initial germination hours. But starch is absent in axis, during 72 hours and 96 hours of germination of seeds. DW treated seeds show better results as compared to control.
- Free soluble metabolites in cotyledons were more during early stages of germination and thereafter it showed marginal a decline, this process showed reverse trend in seedling. Enzyme activity maximum in seedling during later stages of germination; and it was slowly decreased in cotyledons during later stages of germination
- Enzyme activity showed better results in case of seedlings as compared to cotyledons but protease showed different trend in case of ICCC 4, Gujarat – 1 and Gujarat – 2

EXPERIMENT – 2: STUDIES ON GROWTH, DEVELOPMENT, YIELD AND PHOTOSYNTHESIS (Chapter – 3)

Field performance of chickpea cultivars is an important aspect of this study. This study was taken out at two places at Ahmedabad and at Anandper Bhal, which is major chickpea producing area of Gujarat. All the standard agronomic practices were given to the crop during successive period of the crop. Presoaked and control seed were sown. Presoaking treatments with PGRs, which showed better results under laboratory conditions, they were used in the field for study of presoaking. Foliar application of PGRs was also given to the crop at different stages. Foliar applications were given to the crop for three stages, at vegetative, flowering and vegetative + flowering for first year; and for second year, vegetative + flowering stage. Growth and development related parameters on flowering and fruting were observed throughout the crop period. Growth indices, chlorophyll content and nitrate reductase activity were also observed during crop growth. Various photosynthesis related parameters were recorded. Photosynthetic performance measured by Portable Photosynthesis System Cl – 301 CID Inc. (USA) during 9 am to 11 am. Fully expanded leaves were taken out. Maturation studies of seed in relation to morphological, physiological and biochemical was also undertaken. Protein fractions and electrophoretic banding pattern of freshly harvested seed from all treatments were
also performed. Meteorological data (Maximum and minimum temperature, Relative humidity and Rain fall) were collected from Meteorological department.

- The hottest month was May and the coolest month was January. The relative humidity recorded higher values in morning and lower values during January, and there was great variations. Maximum relative humidity was observed in August.
- The maximum temperature and minimum temperature during first year sowing (October 2003) were 35.7 C° and 15.6 C° and there was not much difference in temperature during second and third year of sowing. The maximum and minimum temperature during harvest season (March) did not show major variations.
- The rainfall was maximum in year 2005. But among three years the highest monthly rainfall was in August 2004. In 2005, the rainfall was good in August and September.
- In case of field conditions, presoaked seeds show better emergence as compared to untreated control; Field emergence recorded better results in all treatments under Anandper Bhal field conditions as compared to Ahmedabad field conditions.
- The best stage for foliar application is at vegetative + flowering stage in all four cultivars.
- Growth and development of crop was enhanced by PGRs and phenol. Root length, shoot height, number of leaves, fresh and dry weight of root, shoot and leaves also increased with presoaking and foliar application with PGRs and phenol. All cultivars gave different results in case of various parameters during both years. Number of leaves increased during flowering and initiation of pod, this period is different in all cultivars, in ICCC 4, leaves highly increased during 60 to 75 DAS while incase of Dahod yellow, Gujarat – 1 and Gujarat – 2 it was increased during 45 to 60 DAS with presoaking treatments. Foliar application showed maximum number of leaves at vegetative + flowering stage.
- Reproductive characters like bud, flower, pod and fresh and dry weight of pods were showed good results with PGRs and phenol during both years. GA produced maximum flowers but rate of retained flowers was recorded higher with CA Gujarat – 1 showed maximum retained flowers with presoaking
treatments of DW and CA. In case of foliar application with CA showed maximum retained flowers in Gujarat - 1.

- Chorophyll a, Chlorophyll b, total chlorophyll and carotenoids recorded higher values during flowering and pod formation stage. Presoaking as well as foliar application showed better results as compared to control. It is possible that the chlorophyll synthesis was enhanced by the bioregulators because; the chlorophyllase enzyme which is responsible for chlorophyll depredation might have been inhibited leading to higher accumulation of chlorophyll.

- Leaf area was maximum at 75 DAS in control and presoaking treatments. Foliar application at vegetative + flowering stage showed maximum results.

- Nitrate reductase activity recorded higher values in leaves than shoot. It was increased up to flowering and first stage of pod formation thereafter it showed marginal decline. Foliar application with CA showed highest results in Gujarat - 2 at vegetative (second stage) in leaves and in shoot at vegetative + flowering (first stage). NR activity constitutes the main source of inorganic nitrogen for crop plants and the reduction in activity during senescence is due to the breakdown of proteins.

- Growth indices (RGR of leaf and whole plant, NAR, LWR and LAI) showed better results with treatment as treatments gave second stage result at first stage. NAR is net balance between respiration and photosynthesis. NAR increased as sink activity is increased.

- Yield performance of chickpea is greatly influenced by PGRs and phenols. During first year, various treatments gave better results with PGRs and phenols as compared to control. Foliar application at vegetative + flowering stage showed better results. Presoaking showed good results with GA and CA.

- During second year plant height was maximum in Gujarat - 2 while number of branches recorded more values in Gujarat - 1. Chickpea cultivars showed different results in different parameters.

- During second year number of pods, number of seeds per plant and dry weight of pod and seed was higher in CA 25 ppm soaking treatment in all cultivars. Harvest index and biological yield were highly influenced by PGRs and phenol. Total dry matter was directly related to harvest index.

- Plant growth regulators and phenol as foliar spray or presoaking treatments, increased the yield either by increasing pod setting or seed number.
1000 seed weight is maximum in Gujarat – 2 and minimum in ICCC 4.

In case of ICCC 4 and Dahod yellow harvest index and biological yield were maximum in presoaking treatment of CA, while in case of Gujarat – 1 DW and CA showed best results and in Gujarat – 2 all treatments except foliar application of GA3 showed higher harvest index and biological yield. Improved harvest index is a symbol of increased physiological capacity to mobilize photosynthates from source to sink.

Different cultivars showed different trends in case of photosynthesis related parameters. Highest rate of photosynthesis and PAR was recorded in Dahod yellow. Treatment with CA showed better results. Photosynthetic rate, stomatal conductance and transpiration rate were maximum at flowering stage.

Temperature of leaf and concentration of carbon dioxide was highly influenced by PGRs and phenol.

Photosynthesis active radiation, photosynthesis and stomatal conductance and other photosynthesis related parameters are showed better results during vegetative growth. Photosynthesis rate is highly correlated with chlorophyll and dry matter accumulation. Seed maturation is highly influenced by PGRs.

**EXPERIMENT – 3: STUDIES ON SEED MATURATION**

(Chapter – 4)

Maturation studies of seed in relation to morphological, physiological and biochemical were also undertaken. Protein fractions and electrophoretic banding pattern of freshly harvested seed from all treatments were also performed.

- Morphological characters showed changes in the pattern of seeds, pods and pod wall correlated well with the developmental or maturity stages of seeds. The pod colour changes from green to brown. The seed colour changes from bright green to brownish coffee.

- Physiological characters like seed moisture content and dry matter accumulation had an inverse correlation; moisture decreased gradually with the maturation and dry matter accumulation constantly.

- Biochemical studies showed that there was a positive correlation between accumulation of metabolites and dry matter. Starch, and Protein of the seed
increased, while those of Sugar (total, reducing and non reducing), Amino acid, Phenol, RNA and DNA followed a decreasing trend with the maturity.

- Bioassay of hormones (GA₃, KIN and ABA) showed interesting results. Gibberellins increased sharply and then decreased during later stages. Cytokinins showed fluctuating trend and did not show any definite pattern. Abscisic acid was showed slightly increased during later stages.

- Protein fractions showed interesting results. Different cultivar showed different trend in all fractions. Fractions influenced by treatments. Seeds of chickpea cultivar contain different amount of fractions. Treatments showed improvement in fractions.

- The number and intensities of protein bands showed difference as per cultivars and treatments. Seeds harvested from treated plants, showed higher intensities of colour and number of bands were maximum.

⇒ Salient features:

The silent features of this study are as follows:

1. The required soaking volume and duration for four varieties of chickpea is 1g: 2 ml for 5 hours. Presoaking treatments with PGRs and distilled water show early germination performance and also improvement in SVI. Seedlings from presoaked seeds increased enzyme activities and metabolites in their axis during early hours of germination, which enhanced the earlier germination.

2. Seedling vigour index and quality index highly were influenced by PGRs and phenols.

3. Under laboratory conditions, presoaked seeds gave good results in different concentrations of PGRs. Those concentrations also showed better performance under field conditions and also improved various parameters of growth and yield.

4. In PGRs and phenol treatments, gibberellic acid enhanced highly the growth but caffeic acid regulated the growth.

5. The temperature during the sowing also influenced the seedling emergence. The field performance of crop was best during 2004 – 2005 because the monthly highest rainfall in August 2004, so the residual moisture is high in
the soil. Similarly the crop stand was also good during 2005 – 2006. At flowering stage, the lower temperature is harmful, because it is one of the reason of flower shedding. But at pod setting, the very high temperature, also affected the yield. The crop is highly sensitive to temperature, relative humidity. But foliar sprayed plant and plant raised from presoaked seeds can survive under all conditions and showed better performance.

6. PGRs treatments showed better and early seedling emergence in field during vegetative growth, flowering and final yield. The results of foliar application during different stages of crop; foliar application at both stages that is at vegetative and flowering stage both are superior to other stages.

7. PGRs play important role in sink activity and it also plays role in retaining and abscising reproductive structures

8. Photosynthetic parameters are influenced by different treatments of PGRs and phenols. These are maximum during the early stage of crop and during flowering stage (early pod formation stage) but are lowered at late pod formation stage.

9. The rate of photosynthesis was significantly positively correlated with transpiration rate, stomatal conductance, total dry matter and seed yield.

10. Presoaking treatments with PGRs and phenols showed better results as compared to foliar applications.

11. Harvested seeds accumulated maximum dry weight after anthesis and showed better physiological performance and higher metabolites.

12. Presoaking treatments and foliar application of PGRs and DW increased seed filling capacity in pods, which ultimately led to improved to seed yield.

13. In all cultivars, Gujarat – 2 is early flowering cultivar and ICCC 4 is late flowering cultivar.

14. Gujarat – 2 is bold seeded cultivar and has highest leaf area is highest as compared to other cultivars. Gujarat – 1 has highest numbers in case of branches.

15. Under Anandper Bhal field conditions, the growth, development and yield of crop was best as compared to field conditions of Ahmedabad.