Chapter 1

INTRODUCTION

Water is an essential resource for plant life and metabolism and hence is one of the key inputs to any agricultural system. It is a fundamental constituent of all life, comprising about 90% of the fresh weight in most physiologically active plants. At the cellular level, it is used in chemical reactions as well as to separate and arrange membranes and organelles. At the whole plant level, it is the main carrier for substances traveling among plant organs and tissues. Moreover, because of the large difference in water potential between the hydrated plant cell and dry atmosphere, no other substance in plants is replaced in the same quantities as water. Thus any limitation in the availability of water, generally known as water stress, affects almost all the plant functions.

Water stress is a major factor limiting productivity of pulses in India, since they are cultivated as rainfed crops in most parts of the country. Both chickpea and soybean are severely affected by drought stress at one or the other stages of their growth and development leading to substantial loss of yield potential (Morgan et al., 1991; Desclaux and Roumet, 1996; Egli and Bruening, 2004).

Soybean (Glycine max (L.) Merrill) is an annual legume, native to eastern Asia (China) and has been cultivated for centuries. The leading
producers of soybean include USA, Brazil, Argentina, China and India. In India, it is grown as a ‘kharif’ crop (from late June to October) in northern parts. It occupies an area of 5.7 million ha with average production of 4 million tons in India. The cultivated soybean belongs to the family Fabaceae, sub-family Faboideae, and genus Glycine. The genus presently includes 6 perennial species in the sub-genus Glycine Willd, and two annual species in the sub-genus Soja (Moench) F.J. Herm. The annual species consisting of the cultivated type max and the wild type Soja Sieb. and Juce., have a chromosome number of $2x = 40$ and can be readily inter-crossed. Soybean occurs in various sizes, and in several hull or seed coat colour, including black, brown, blue, yellow and mottled. The chemical composition of seeds consists of proteins (40%), oil (20%), 5% (ash) and carbohydrates (35%). The principal soluble carbohydrates, saccharides of mature soybeans are the disaccharide sucrose (range 2.5-8.2 %), the trisaccharide raffinose (0.1-1.0%) composed of one sucrose molecule connected to one molecule of galactose, and the tetrasaccharide stachyose (1.4 to 4.1) composed of one sucrose connected to two molecules of galactose. Oligosaccharides raffinose and stachyose protect the viability of the soybean seed from desiccation. Soybeans are a source of complete protein. A complete protein is one that contains significant amounts of all the essential amino acids that must be provided to the human body because of the body’s inability to synthesize them. For this reason, soybean is important to many vegetarians. Soybean protein is similar to that of other legume seeds, but has the highest yield per square meter of growing area, and is the least expensive source of dietary protein.

Rainfall is the principal source of water for most of the world’s soybean production. Reports of seasonal soybean water consumption vary from about 250 mm under a dry situation to approximately 840 mm under conditions of continuous, optimal water availability. During soybean germination, the water content of soil should not exceed 85% or be less
Introduction

than 50% (Doorenbos and Kassam, 1986). To achieve maximum yield, an adequate supply of water must be available during the critical seed-development period. A moisture deficit during the pod-filling period is more detrimental to yield than a deficit during flowering (Sionit and Kramer, 1977). In soybean also, it has been reported that water stress during pod-filling period reduces yield by 5-38% and seed size by 11-35%, but the seed number was not affected. It is probably due to the accelerated leaf senescence which results in shortening of seed filling period (Egli and Bruening, 2004).

Chickpea (Cicer arietinum L.) is one of the earliest grain crops cultivated by man and has been found in Middle Eastern archaeological sites dated 7500-6800 BC (Zohary and Hopf, 2000). Today, chickpea continues to play an important role in agricultural systems, ranking third behind the dry bean (Phaseolus vulgaris L.) and pea (Pisum sativum L.) in terms of world grain legume production. It is an annual species that originated in southwestern Turkey (Ladizinsky, 1975). It exists as two groups based on seed size, ‘macrocarpa’ and ‘microcarpa’. The ‘macrocarpa’ are also known as ‘Kabuli’ (large, rams-head shaped and light brown seeds) and ‘microcarpa’ as ‘Desi’ (small, angular and dark brown colored seeds) by plant breeders in Indian subcontinent. Cicer, which was classified under tribe Vicieae Alaf, was later reported to be a monogeneric tribe, Cicereae (Kupicha, 1981), which differs from the related genera in Vicieae by its glabrous style, inflated pods and glandular pubescence. The genus includes 9 annuals and 34 perennial herbs (Van der Maesen, 1972; Muehlbauer, 1993). Chickpea is a self-pollinated crop and cross-pollination is rare: only 0-1% is reported (Smithson et al., 1985). Although spoken as a day neutral, chickpea is a quantitative long day plant and produces flowers in every photoperiod (Smithson et al., 1985). Chromosome number in Cicer species can be generalized as 2n = 2x = 16, although varying numbers both for chickpea (2n = 2x =14, 16, 24,
32, 33) and other wild species (2n = 2x = 14, 16, 24) have been reported but could not be confirmed by other workers.

Chickpea is grown in tropical, subtropical and temperate regions. ‘Kabuli’ is grown in temperate region while ‘Desi’ type chickpea is grown in semi-arid tropics (Muehlbauer and Singh, 1987; Malhotra et al., 1987). India is the largest producer of chickpea (6.2 x 10⁶ t), accounting for 74% of the total world production (8.8 x 10⁶ t) and reflecting the importance of chickpea as a protein source in the diet of people in developing countries. In India, it is grown as rainfed, post rainy season crop especially in northern parts of the country. It is an important winter season food legume of Punjab and is cultivated in about 13000 hectares with an annual production of about 10000 tons (Singh et al., 1990; Singh and Ocampo, 1993). It is valued for its nutritive seeds with high protein content (25.3-28.9%) as well as 38-59% carbohydrates, 3% fibre, 4.8-5.5% oil, 3% ash, 0.2% calcium and 0.3% phosphorus (Hulse, 1991).

Water stress affecting various physiological and biochemical functions involved in plant growth and maintenance develops, when the cellular water content drops and plants are unable to derive from soil to maintain the required level of cellular water content (Misra et al., 2002). Water deficit inhibits growth as leaf area, leaf development, root density and depth, reduces physiological and metabolic processes, limits hydraulic conductance of xylem vessels in roots, retards photosynthesis, carbon dioxide uptake and carbohydrate synthesis, nitrogen metabolism (Misra et al., 2002).

The effects of water stress depend on the timing of the water deficits in relation to phenology of the plant. During early vegetative growth, seedling growth, leaf development will be inhibited leading to reduction in carbon gain throughout the growing period because of reduced canopy development (Nilson and Orcutt, 1996). This is
accompanied by other secondary effects like reduced uptake and altered distribution pattern of the nutrients. Moderate, short-term water deficits that occur during vegetative development, before fruit formation or podding, generally do not affect the yield significantly. These, however, will reduce plant size, which delays or precludes full canopy closure and full sunlight interception.

The sensitivity of plants to water deficit is particularly acute during the reproductive development because reproduction involves several processes that are extremely vulnerable to a change in plant water status (Saini, 1997). Stress during this phase can delay or completely prevent flowering, both through inhibition of floral induction and development. It can cause loss of pollen fertility, failure of pollination and fertilization, zygotic abortion and impaired embryo development. The entire reproductive phase can be divided into number of substages, including floral initiation, differentiation of various parts of an inflorescence and/or flower, male and female meiosis, development of pollen and embryo sac, pollination, fertilization and seed development. Drought occurring during each of these substages has quite specific effects, all of which lead to decline in yield (NeSmith and Ritchie, 1992; Jamieson et al., 1995). Substantial losses have been recorded in soybean and chickpea experiencing drought stress during reproductive growth (Morgan et al., 1991). No studies exist in legumes on flower development, fertilization and embryogenesis in response to water stress and these are least understood aspects in Soybean and Chickpea under stress. Also, the mechanism by which water stress inhibits flower induction or accentuates flower and pod abscission remains obscure. Stress during fruit maturation inhibits the seed filling as well as results in abscission of fruits (O'Toole and Chang, 1979).

The effects of water deficits on reproductive growth have been worked out in cereals (Saini and Westgate, 2000) while information is
lacking in legumes. In cereals, every stage right from flower initiation up to kernel maturation is affected by drought stress (Wopereis et al., 1996; Winkel et al., 1997). Gametophyte development is also impaired resulting in loss of pollen fertility (Sheoran and Saini, 1996). Comparatively few attempts have been made if female infertility also contributes to the decline in yield in response to drought and this aspect needs thorough study of development and functioning of megagametophyte during stress. Water deficit during podding and seed set can also be quite detrimental to yield because pods abort, or if the stress occurs after pods partially elongate, seeds will abort within pods. Water deficit stress during early pod development and seed filling is more detrimental. The yield loss is due primarily to a decrease in pod number per plant (Momen et al., 1979), and reduced seed-filling period, which results in reduction of 50% in case of chickpea and upto 80% in case of soybean (McWilliams et al., 1999). Whether these responses are direct effect of desiccation or a consequence of reduced photosynthesis is speculative. Water deficits have a direct effect on the water status and development of reproductive structures. Low water potential may decrease sink intensity of developing pods by inhibiting expansion growth or assimilate metabolism. Nutrient deficiency, limitation in assimilates supply, hormonal imbalance, and correlative inhibition all have been implicated in pod abortion and abscission (Peterson et al., 1990; Wiebold, 1990). Lack of water during early seed-filling exerts its effect on the seed’s sink potential (Mambelli and Setter, 1998). The endosperm cell number in cereals is sensitive to environmental conditions during cell division and hence the reduction in yield under drought during early seed development is mainly due to a lesser number of endosperm cells. Once the sink potential of the seed is established, the process of starch and protein accumulation starts which can be reduced by drought by limiting the rate and duration of reserve deposition (Saini and Westgate, 2000). Grain filling gets dependent on remobilization of assimilates under stress conditions and has also been
investigated in chickpea (Leport et al., 1999). The reallocation of carbon to grain not only depends on the amount of carbohydrates stored in the seed but also on the ability of the seed to incorporate the re-translocated carbon in the embryo or endosperm. Various enzymes involved in the starch and protein synthesis, amino acids' metabolism and sucrose hydrolysis require to be examined in relation to water stress to understand the seed filling during stress situation. Genotypic variation in expression of these enzymes needs to be explored to find out the reasons underlying poor seed development under stress.

Restriction of photoassimilates towards developing reproductive sinks (flowers, pods and seeds) has been observed under water deficit stress (NeSmith and Ritchie, 1992). This effect has been attributed to depletion of cytokinins (CKs) due to reduction in its flow and biosynthesis under stress (Pospíšilová et al., 2000). CKs synthesis and/or transport from the roots get reduced during the drought. Flower and pod set are regulated by the supply of assimilate to developing flowers and pods (Schou et al., 1978). CKs influence the movement of assimilates, a phenomenon known as cytokinin-induced nutrient mobilization. Probably CKs cause nutrient mobilization by creating a new source-sink relationship. Nutrients translocated in the phloem move from a site of production or storage (the source) to a site of utilization (the sink) (Taiz and Zeiger, 2003). As during water deficit, CKs synthesis and/or transport are decreased, its exogenous application to flowers and fruits is expected to increase their sink strength and thereby improve crop productivity (Gifford and Evans, 1981; Gifford et al., 1984; Lutze and Gifford, 2000). CKs rescue the flowers and pods by redirecting the movement of assimilates into treated tissues, increasing sink strength and subsequent growth rates and preventing abscission of the developing flowers and pods (Dybing, 1994; Reese et al., 1995).
Most of the work done on chickpea and soybean pertains to the effect of drought on vegetative growth and yield traits (Frederick et al., 1989; Singh, 1991; Morgan et al., 1991; Cho et al., 2002) and less information is available on drought effects on different stages of reproductive phase especially development and functioning of gametes. Understanding drought effects on various reproductive phases will lead to identification of causes of reproductive failure that could be developed as finer traits linked to drought sensitivity. Such traits and markers can be useful in selection and breeding studies for stress tolerance. Keeping in view these, the present work was devised with the following objectives:

**OBJECTIVES**

- Evaluation of water deficit stress induced damage to reproductive growth at various organizational levels
- Comparative assessment of sensitivity of various reproductive stages to stress
- Identification of tolerance-related mechanisms/traits
- Investigation of cytokinin’s role in stress responses.