CHAPTER II
LITERATURE REVIEW

1. Peanut and the risk for water stress

Peanut (*Arachis hypogaea* L.) is an important cash crop legume and a rich source of oil (40-50%) and protein (20-40%), but it is low in percentage of carbohydrates (10-20%) (Savage and Keenan, 1994; Maiti, 2002). Most peanut production in many parts of the world especially in Asia has been used for peanut oil and the small portion has been used for other human consumptions such as roasted, boiled or salted. The unique morphological feature of peanut is aerial flowering that gives rise to subterranean pods. Species of genus *Arachis* are perennial or annual legumes. There are about 70 species, most of them are diploid ($2n = 2x = 20$ or 18) and two species are allotetraploid with $2n = 4x = 40$. Five species have been cultivated, but only *A. hypogaea* ($2n = 40$) has been domesticated and grown extensively (Stalker, 1992). On the basis of reproductive and vegetative branching and on pod morphology, this species is classified into two subspecies and six botanical varieties (Krapovickas and Gregory, 1994). The runner and Virginia market-types are genetically based primarily on var. *hypogaea* with some introgression of germplasm from vars. *fastigiata* and *vulgaris*, but the valencia market-type is derived exclusively from var. *fastigiata* and the Spanish market type from var. *vulgaris* with some introgression from *A. monticola* Krapov and Rigoni (Isleib et al., 2001). In the USA, four market types are of greatest economic importance where approximately 70 % of the peanuts grown are small-seeded runner types, while 20% are large-seeded Virginia types, 10 % are Spanish, and less than 1 % are valencia market-type (Knauft and Gorbet, 1989). Peanut is cultivated worldwide in tropical, sub-tropical, and warm temperate areas. Peanut production area worldwide is 19.3 million ha in about 82 countries. More than half of the production area occurs in Asia, followed by Africa and the Americas. China, India, and the USA are the largest producers in the world. About 80 percent of world's peanut production is under rain-fed agriculture system where drought is the major constraint limiting

crop productivity (FAOSTAT, 2008; Wright and Nageswara Rao, 1994a). In this area year-to-year variability of yield also indicates the severity of limited water.

2. Timing and period of drought on peanut

Low and unpredictable rainfall is usually perceived to be the most important factor of drought resulting in low yields in many parts of the world. In rain-fed regions, drought seems to be the major abiotic stress in peanuts (Johansen et al., 1994). Peanut is frequently subjected to drought stresses of different duration and intensities. The duration and intensity of drought, and the growth stage at which the stress occurs have large effects on the amount of yield reduction in peanut (Wright and Nageswara Rao, 1994a). For example, peanut productivity dependent on natural rainfall is prone to intermittent drought during vegetative and reproductive growth periods. Intermittent drought stress at pod setting can reduce yield substantially (Nautiyal et al., 1999), and yield loss of 15 to 88 % has been reported (Ravindra et al., 1990; Nageswara Rao et al., 1989; Vorasoot et al., 2003). Early season drought is defined as a single event drought occurring during the vegetative or pre-flowering phase (Nageswara Rao et al., 1989). Drought during the vegetative phase or pre-flowering stage has a small effect on yield or some case was found to increase yield (Nageswara Rao et al., 1985, 1988; Nautiyal et al., 1999). Terminal drought, which occurs during the pod-filling phase of peanut, is common and is a serious limitation for yield under rain-fed conditions (Nageswara Roa et al., 1985).

3. Drought resistance mechanisms

Various drought resistance mechanisms exist reflecting the plasticity of plant responses to difference water stress patterns under diverse environments. Thus, the first step in designing strategies to alleviate drought stress is the characterization of the drought-resistant mechanisms for the target environments. In literature reported herein, drought-resistant mechanisms can be defined as (i) drought escape, (ii) drought resistance, and (iii) drought tolerance.

3.1 Drought escape

Drought escape is a mechanism of matching phenological development with the period of soil moisture availability to minimize the impact of drought stress on crop production in environments where the growing season is short and terminal drought predominates (Turner, 1986a). This mechanism is an important strategy especially for crops grown during a post-rainy season and reliant on stored soil moisture. Plants that have a drought escape mechanism can complete their life cycles during the wet season before the onset of drought. Drought escape cultivars should be of early maturity to better match seasonal soil moisture viability in order to escape drought compared to later maturing cultivars. For example, at the ICRISAT, considerable progress has been made in shortening crop duration without unduly penalizing yield potential (Subbarao et al., 1995). For many crop species, breeding for early maturity and high-yielding genotypes is a major objective, not only to match phenology to season length but also for fitting genotypes into more intensive cropping systems (Nageswara Rao and Nigam, 2003).

3.2 Drought resistance

Drought resistance can sometime be referred to as dehydration avoidance or drought avoidance with high water potentials, and is the ability to maintain tissue hydration consisting of two major mechanisms; maximizing water uptake through improving the capacity of the root system to acquire water, and optimization of the use of water for dry matter production (Subbarao et al., 1995).

Root attributes to uptake water: Root size, depth, length, density, and hydraulic conductance are basic to meet the transpiration demand of the shoot, and are major attribution for improving water uptake (Passioura, 1982). Large root systems are considered to be a major trait for improving water uptake. Since osmotic adjustment allows continued root growth at low water potentials, attribution of root allows the plant to explore a greater volume of soil for water at depth (Turner, 1986a). Root responses when soil moisture dries out are also important mechanisms for drought avoidance (Ketring, 1984; Songsri et al., 2008b). In addition, the ability to extract soil water has been related to improved drought resistance in peanut. Peanut genotypes with large root systems, deeper rooting depth, and high root to shoot ratio

can maintain high plant water status and gave high yield under water stress (Rucker et al., 1995; Songsri et al., 2008b). The depth of rooting is an important character under rain-fed environments. Even though terminal drought stress is common for many post-rainy season legumes, crops are not necessarily limited by a deficiency of the crop to fully extract it fast enough for yield performance (Jordan et al., 1983). Root hydraulic conductivity and root length density are important factor influencing water uptake by plants. Root length density usually decreases exponentially with depth (Wiebe, 1980). Plant with high root length density should be able to rapidly extract water without difficulty (Passioura, 1983). A decrease in root hydraulic conductivity could help in conserving soil moisture early in the season, so that it is available for grain filling (Passioura, 1983). Root hydraulic conductivity should be beneficial in environments where grain yield depends on the amount of available water left in the soil at the onset of flower, this can stabilize yield when drought occurs after flowering (Passioura, 1972).

Shoot attributes to reduce water loss: Dehydration avoidance in shoots also plays an important role in regulating water use of crop under water stress. Canopy structure comprising leaf size, leaf shape, leaf surface characteristics and reflectance properties, leaf angle, and the geometrical arrangement of leaves in the canopy, are important characters controlling light extinction coefficient and radiation use efficiency of the crop. Canopy structure could also control water loss in plant (Subbarao et al., 1995). Other mechanisms reducing water loss, such as decreasing stomatal conductance, leaf rolling, leaf movement, increased reflection and a decrease in leaf area are the processes that may increase productivity under water stress (Subbarao et al., 1995; Turner, 1986a, b). However, stomatal closure is a powerful tool for the reduction of water loss and appears to be under the control of both abscisic acid (ABA) and cytokinins (Turner, 1986a; Taiz and Zeiger, 2006). Additionally, the vapour pressure deficit of the air has an effect on the stomatal conductance independent of any changes in leaf water potential (Turner, 1986b).

Osmotic adjustment: The major mechanism of drought resistance is osmotic adjustment (Turner, 1986a). Osmotic adjustment can be defined as the active accumulation of solutes such as amino acids, sugars and ions within the plant tissue in response to a lowering of soil water potential (Morgan, 1984). As soil dries, plant

roots that have osmotic adjustment mechanism can continue to absorb water by decreasing their water potential in the cell without an accompanying decrease in turgor. Osmotic adjustment should not be confused with the increase in solute concentration that occurs during cell dehydration. Osmotic adjustment provides the driving force for extracting water from low water potential, and plays an important role in determining the drought resistance of given genotypes (Taiz and Zeiger, 2006). Osmotic adjustment has been observed to maintain stomatal opening and photosynthesis (Turner, 1986a, b), to lower leaf water potentials, and to defer leaf rolling and leaf death (Hsiao et al., 1984). The ecological habitat of plant, the growth stage, and growth conditions can influence the degree of osmotic adjustment (Morgan, 1983; Blum and Sullivan, 1986; Girma and Krieg, 1992).

3.3 Drought tolerance

Drought tolerance is the ability of plant cells to function while dehydrated, it is also known as drought tolerance with low water potentials (Taiz and Zeiger, 2006). Drought stress results in cell disruption, if plants cannot maintain water in the cell through dehydration avoidance mechanisms; membrane disorders may occur due to lipid peroxidation or structure changes (Blum, 1988). Leakage of solutes as a consequence of membrane damage is a common response of groundnut tissue to drought stress. Plants differ in dehydration tolerance and an important factor for such differences depends on the capacity of the cell membrane to prevent electrolyte leakage, and denaturation of protein to maintain cell membrane stability at decreasing water content (Tripathy et al., 2000).The ability of maintenance of nuclear integrity is essential for drought tolerance (Boyer, 1983). Plants that have drought tolerance or dehydration tolerance can continue metabolism in cells at low water potential (Santarius, 1967). Most crops belonging to the drought-tolerance category, generally, have poorly developed drought-avoiding mechanisms and are usually associated with slow rates of growth and development (Bewley, 1979; Ludlow, 1980). Critical relative water content is the most meaningful index for identifying legumes with differences in drought-tolerant mechanisms (Sinclair and Ludlow, 1986).

4. Terminal drought and its effects

The growth stages which are particularly sensitive to drought have been identified. Moreover, in the years when the rainy season ends early, crops must depend on stored soil water reserves to meet evaporative demand which is common for terminal drought stress. For instance, in rice-based cropping systems without irrigation, peanut are often grown in the dry season and are solely dependent on soil water reserves. Therefore, these peanuts are often exposed to terminal drought. Drought stress occurring during the seed-filling phase or terminal drought has been observed to cause the greatest reduction of pod yield (Nageswara Rao et al., 1985; Ravindra et al., 1990; Wright et al., 1991).

4.1 Vegetative growth and yield

Under water deficit conditions, pod yield was affected by decreasing pod growth and development (Reddy et al., 2003; Chapman et al., 1993c), and drought also decreased the number of mature pods and pod yield (Nautiyal et al., 1999). The greatest reduction in kernel yield occurred when stress was imposed during the seed-filling phase (Nageswara Rao et al., 1985). Similar, yield depressions in response to late season drought in peanut have also been observed elsewhere (Pallas et al. 1979; Wright et a. 1991). For late-season drought in the longer season, Virginia types reduced pod yields more severely than did Spanish types (Wright et a. 1991). Nageswara Rao et al. (1989) reported that the peanut cultivar Robut 33–1 gave 13–19 percent yield increase when drought stress occurred before flowering, but showed 56–83 percent yield reduction when drought stress occurred during flowering and pod and seed development stages. Water deficits during pod filling generally reduced pod weight, seed weight and harvest index (Chapman et al., 1993a; Nautiyal et al., 1999; Reddy et al., 2003). Water deficits during kernel or seed development reduce pod and seed weight (Chapman et al., 1993a; Nautiyal et al., 1999; Reddy et al., 2003).

4.2 Morphological and physiological process

Drought stress can significant affected physiological processes i.e. plant water status, stomata resistance (Wright and Nageswara Rao, 1994a) and photosynthesis (Williams and Boote, 1995). Metabolic changes in response to water stress include

reduction in photosynthetic activity (Ritchie et al. 1990). Terminal drought affected leaf area index, relative water content and transpiration at about 2 weeks after the occurrence of water deficit was apparent in the soil (Clavel et al., 2004). Clavel et al., 2004 observed that the water treatment effect was also observed at 64 DAS for transpiration rates as well as the closely related measurement of stomatal conductance. They also found that water treatments have no effect on the partitioning coefficient. Considering that, partitioning coefficient is a more reliable selection criterion for identifying genotypes tolerant to end-of-season drought than yield (Ndunguru et al., 1995). Collino et al. (2001) observed that response mechanisms to drought stress at pod-filling phase of two peanut cultivars were different. The cultivar Manfredi 393 INTA had the ability to extract more water while the cultivar Florman INTA developed faster and produced more pods. Under water stress regime, pod water use efficiency was significantly reduced in both cultivars.

4.3 Seed quality

Terminal drought is common and can affect both yield and quality. Drought provides favorable conditions for pest and disease infestation in peanut, especially the diseases caused by various kinds of fungi (Wright and Hansen, 1997). Among these fungi, *Aspergillus* spp. are most important considering their ability to produce toxic compounds known as aflatoxins. Aflatoxins deteriorate the quality of peanut kernels and its products rendering them unfit for human and live stock consumption and cause economic and trade problems at almost every stage of marketing of peanut especially during export. Aflatoxin contamination is a major problem in peanut kernels infested by *A. flavus* particularly under terminal drought conditions (Diener et al. 1987). Total oil content was not affected by early-season drought (Conkerton et al., 1989; Bhalani and Parameswaran, 1992) but declined (by up to 3%) under mid-season (50-80 days after sowing (DAS) drought (Conkertón et al., 1989). For late-season drought (110-140 DAS), different studies have reported no effect (Conkerton et al., 1989; Musingo et al., 1989) and a decline (Bhalani and Parameswaran, 1992) in total oil content. However, Dwidevi et al., 1996 found that terminal drought significantly reduced total oil, and linoleic and behenic fatty acid content, and significantly increased total protein and stearic and oleic fatty acid content. Drought during pod development can

decrease seed germination in peanut. High germination was obtained when the average soil water tension was maintained at less than 0.6 bars. On the other hand, peanut under soil water tension greater than 15 bars has been observed to produce seed with germination of only 5 to 22 % (Pallas et al., 1977).

5. Aflatoxin and occurrence

Aflatoxins, toxic secondary metabolites, are well recognized as potent carcinogenic, teratogenic and immunosuppressive substances (Turner et al. 2000, Wild and Hall 2000, Hall and Wild 2003) produced when toxigenic strains of the fungi *Aspergillus flavus* Link. ex Fries and *A. parasiticus* Speare grow on peanuts subjected to drought (Blankenship et al. 1984). Aflatoxin contamination of peanut was first recognized as a serious problem following the outbreaks of 'turkey X disease' in the United Kingdom in 1960 (Lancaster et al., 1961; Sargent et al., 1961). In the past, aflatoxin was considered predominantly a postharvest problem and as such, received little attention in crop improvement programs. Aflatoxins are a group of closely related compounds with small differences in chemical compositions based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography. The four major aflatoxins are called B_1, B_2, G_1, and G_2 (Ellis et al., 1991; Shapira et al., 1996; Bennett and Klich, 2003). Other significant members of the aflatoxin family, M_1 and M_2, are oxidative forms of aflatoxin B_1 and B_2 modified in the digestive tract of some animals and isolated from milk, urine and feces (Squire, 1989). Drought stress and high soil temperatures for 3 to 6 weeks before harvest were shown to be the primary contributing factors for aflatoxin contamination in peanut (Blankenship et al., 1984; Hill et al., 1983; Sanders et al., 1985; Wilson and Stansell, 1983). Damaged pods infested by plant pest i.e. the lesser cornstalk borer (*Elasmopalpus lignosellus*) or root-knot nematodes (*Meloidogyne* spp.) had a higher incidence of *A. flavus* and aflatoxin concentrations than undamaged pods (Lynch et al., 1990; Timper et al., 2004). Timper et al. (2007) demonstrated that root galling, even in the absence of pod galling, can increase aflatoxin contamination of the peanut kernels. Nematode infection of roots causes physiological changes in the plant which may increase its susceptibility to infection by *A. flavus* or aflatoxin contamination.

5.1 Risk for *A. flavus* infection and aflatoxin production

5.1.1 Environmental factors

Aflatoxin production, like most quantitatively-controlled traits, is influenced by the environment. Identification of environmental factors that affect aflatoxin production is important in determining their potential influence on aflatoxin contamination. The major environmental factors that influence aflatoxin production in peanut are discussed individually, as a basis for assessing their relative importance. Aflatoxin production in peanut appears to be under genetic control that is quantitative in nature. The environmental factors affecting pre-harvest aflatoxin incidence in peanut crops are reasonably well understood, and increased incidence is associated with both drought stress and high soil temperature (Blankenship et al., 1984; Hill et al., 1983; Sanders et al., 1985; Wilson and Stansell, 1983; Sanders et al., 1993; Wright and Hansen, 1997; Nageswara Rao et al., 2002, Horn, 2005). Late season drought stress, particularly in the semi-arid tropics, is a major factor associated with aflatoxin contamination (Mehan, 1987). Neither heat nor drought alone can induce high levels of preharvest contamination. Lower soil temperature was found to reduce aflatoxin contamination in peanut (Hill et al., 1983). *Aspergillus spp.* are uniquely thermo-tolerant, and thrive during drought and heat stressed conditions. The temperature optimum for growth of *A. flavus* (25-42°C) is higher than for many other species (Klich et al. 1992). Gqaleni et al. (1997) found that temperatures between 25 to 30 °C and water stress are the most favorable conditions for *Aspergillus* growth. Associations between temperature and kernel colonization by *A. flavus* and subsequent aflatoxin contamination have been observed under controlled greenhouse conditions (Payne et al. 1988; Thompson et al. 1980) and in field studies (Jones et al. 1980; Zuber et al. 1983). Increased aflatoxin contamination was observed in drought-treated peanuts with increased soil temperatures (Cole et al., 1985). The optimal temperature for production of aflatoxin is approximately 30°C (Sorenson et al., 1967). Dorner et al. (1989) also concluded that a higher soil temperature favors *A. flavus* growth and aflatoxin production. The field environment provides the basis for identification of causes, of poor plant health, reduced vigor, and other symptoms of abnormal development expressed by the growing plant. In Australia, more than 60% of peanuts are grown under seasonally rain-fed conditions in Southeast Queensland,

where there is a high probability of end-of-season drought and associated risk of aflatoxin contamination (Wright and Hansen, 1997). In general, end-of-season drought and elevated soil temperatures enhance aflatoxin production.

5.1.2 Plant physiological factors

Peanut kernels become susceptible to *A. flavus* invasion when kernel moisture content is below 30% (McDonald and Harkness, 1967). Kernel moisture in a range 15 to 30 % is at risk to aflatoxin contamination. Pod moisture adjusted to 20% resulted in higher kernel colonization than at 25% kernel moisture in peanut (Mixon and Rogers, 1973; Mixon and Rogers, 1975). The greatest seed colonization in peanut has been recorded at water activity (a_w) of 0.92-0.96 at temperatures of 22 to 37 °C (Horn, 2005). *Aspergillus* spp were found to be prevalent at kernel moisture contents and a_w values below 14% and 0.73, respectively. It has also been found that the minimum a_w required for fungal growth and aflatoxin production are 0.78 and 0.83, respectively (Lacey et al., 1991). Mature and large kernels are more tolerant to aflatoxin contamination than smaller and immature kernels (Cole et al., 1989; Hill et al., 1981). Invasion by *A. flavus* and aflatoxin contamination in peanut subjected to drought stress usually occur to a greater degree in damaged, small and immature kernels (Cole et al., 1985; Dorner et al., 1989; Hill et al., 1983; Sanders et al., 1985). Mehan et al. (1986) showed that the levels of *A. flavus* and aflatoxin B_1 were higher in seeds from over-mature pods than immature and mature pods, especially under drought stressed conditions. Pods with damaged shells are more likely to contain toxic kernels than are pods with undamaged shells (McDonald and Harkness, 1967; Porter and Smith, 1974; Subramanyam and Rao, 1977).

5.1.3 Plant biochemical factors

Peanut kernels are a good source of sugars and amino acids which are used for fungal growth and aflatoxin synthesis. Aflatoxin production is induced in the presence of simple carbohydrates especially by glucose, sucrose, fructose and maltose (Abdollahi and Buchanan, 1981; Buchanan and Lewis, 1984; Buchanan and Stahl, 1984; Feng and Leonard, 1995). Under drought conditions, complex carbohydrates and proteins are broken down by enzymes into simpler sugars and amino acids,

respectively (Pandey et al. 1984). Various kinds of sugars have been identified in the peanut kernel; the major sugars are sucrose, glucose and fructose, raffinose, stachyose, verbascose and ajucose (Basha, 1992; Marshall et al., 1991; Oupadissakoon et al., 1980; Pattee et al., 2000). The amino acids that have been identified in peanut are aspartic acid, threonine, serine, glutamic acid, proline, glucine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, ammonia and arginine (Beuchat et al., 1975; Singleton et al., 1996; Young, 1980). Genotypic variation has been observed in peanuts for sugars and amino acids (Grimm et al., 1996).

Several *in vitro* studies have indicated that fatty acid composition could either directly or indirectly affect aflatoxin contamination (Fabbri et al., 1983; Passi et al., 1984; Doehlert et al., 1993; Burow et al., 1997a). In different studies, linoleic acid reportedly increased or decreased *Aspergillus* development and aflatoxin production (Passi et al., 1984; Doehlert et al., 1993; Calvo et al.,1999). Holbrook et al. (2000b) evaluated the effect of altered fatty acid composition on PAC in peanut, but observed no measurable effect of reduced linoleic acid composition on PAC. They concluded that the products of the lipoxygenase pathway that have been shown to affect aflatoxin biosynthesis *in vitro* may not be present in sufficient quantities to affect aflatoxin contamination of developing peanut seeds.

Ingram et al. (1999) observed that *A. flavus* populations increased on peanut root and pod surfaces under dry soil conditions. Puntase et al. (2004) observed that water deficit promoted more exudation of sucrose, and it may support *A. flavus* colonization under stress conditions. Under drought and heat stress, the losses of phytoalexins production capacity, an immune system, led to aflatoxin production. The ability to maintain pod and plant moisture contents under drought stress is the main mechanism that can help to maintain the capacity of plant to produce stilbence phytoalexin preventing PAC (Dorner et al., 1989; Wotton and Strange, 1987). Latha et al., (2007) found that aflatoxin production was negatively correlated with total phenols in kernels, so that peanut seeds with high phenols should have low aflatoxin contamination.

6. Reducing of preharvest aflatoxin contamination through drought resistance

Holbrook et al. (2000a) evaluated the resistance to PAC in genotypes previously reported to have varying levels of drought tolerance, and concluded that tolerant genotypes also had greatly reduced aflatoxin contamination. Under drought stress, the loss of the capacity of peanut seeds to produce phytoalexins, an immune response to counteract fungal colonization resulted in higher aflatoxin contamination. The ability to maintain higher pod moisture contents during drought periods may be importance traits enabling cultivars to resist aflatoxin production (Dorner et al. 1989; Wotton and Strange 1985). Researchers have shown a relationship of increased drought tolerance (canopy temperature, visual stress rating, and water use efficiency) and reduced aflatoxin production (Cole et al. 1993; Holbrook et al. 2000a; 2008; 2009). Thus, physiological traits could help breeder to reduce aflatoxin contamination in peanut. Arunyanark et al. (2009a) found significant relationships between aflatoxin contamination and specific leaf area (SLA), root length density (RLD), and chlorophyll density (ChlD) under long term drought in peanut but they did not focus on terminal drought which is the most important period for aflatoxin contamination. In Australia, the drought-tolerant genotype Streeton showed lower levels of aflatoxin compared to other commercially grown varieties (Cruickshank et al., 2000). This cultivar has up to 40 percent lower aflatoxin levels during the years of high aflatoxin incidence in comparison to other cultivars.

7. Genotypic variation of peanut

7.1 Genotypic variation in *A. flavus* resistance

A. flavus resistant cultivars should be a component of an integrated program of aflatoxin management. Most sources of resistance to *A. flavus* in peanut show low levels of resistance or tolerance. Such partial resistance is presumably governed by polygenes and is assumed to be similar to horizontal resistance (Fry, 1982). Resistance to invasion and colonization by *A. flavus*, located in the seed coat, was suggested to be an effective means of preventing aflatoxin contamination. Differences have also been reported for the ability of peanut seeds to support the production of aflatoxins (Aujla et al., 1978; Nagrajan et al 1973; Rao et al., 1967; Tulpule et al., 1977). Screening of peanut genotypes for resistance to *Aspergillus* spp. infection was

done in the laboratory and indicated possible resistance to aflatoxin contamination/production (Azaizeh and Pettit, 1986; Azaizeh and Pitt, 1987; Bartz et al., 1978; Kushalappa et al., 1979; Mixon and Rogers, 1975). However, the identified resistant genotypes to *Aspergillus* invasion failed to show such resistance when tested under glasshouse or field conditions (Mehan et al., 1989; Sanders et al., 1985). Some studies have, however, shown consistent resistance or susceptibility for tested genotypes (Mehan et al., 1986; Nehdi, 1989). Mixon and Rogers, (1973) investigated *A. flavus* infection on rehydrated sound mature seeds of peanut inoculated with conidia of *A. flavus* in an environment favorable to fungus development. They found that two germplasm lines, PI 337409 and PI 337394F, were found to be resistant to seed invasion and colonization by *A. flavus*. The genotypes J-11 and Lampang were resistant to fungi when tested under both under dry and moist conditions (Kisyombe et al., 1985). The AR-1, AR-2, AR-3 and AR-4 have shown resistance to aflatoxin production compared to other cultivars and germplasm (Mixon, 1983; Mixon, 1986). The early maturing and popular cultivar in India, JL-24, is susceptible, whereas J-11 which is also widely grown in western and central India is resistant to *A. flavus* invasion (Upadhyaya et al., 2001). Among 25 breeding lines tested in West Africa the lines 55-437, J11 and PI 337394 were the least infected. The trials conducted at ICRISAT shown that ICGV 87084, ICGV 87094 and ICGV 87110 were resistant to *A. flavus* invasion (Waliyar et al., 1994).

7.2 Genotypic variation in drought resistance

A large genetic variation in peanut has been reported for yield and the model component traits i.e. water transpired by crop (T) (Wright et al., 1994; Nageswara Rao and Wright, 1994), transpiration efficiency (TE) (Wright et al., 1994; 1988), and harvest index (HI) (Nageswara Rao et al., 1992). Root penetration into deeper soil layer is a function of both genotype and environment; interaction between the two often makes it difficult to distinguish genotypic differences in root growth. Substantial genotypic variation in rooting depth, root volume and water extraction pattern at different depths has been reported (Ketring, 1984; Mathews at al., 1988a; Wright et al., 1991; Chapman et al., 1993a). Drought resistance may be enhanced by improvements in soil water extraction capability (Wright and Nageswara Rao, 1994a;

Maiti et al., 2002) that can improve water use efficiency (Hebbar et al., 1994). Rucker et al. (1995) evaluated drought tolerance characteristics of 19 peanut genotypes that differed in the size of their root systems. Under drought conditions, peanut genotypes differed in shoot dry weight, root to shoot ratio, canopy temperature and visual stress rating. Tifton–8 and PI 315628 had the lowest visual stress and temperature rating, and PI 315628 also had the largest root system measured.

Researchers have investigated the inheritance of aflatoxin traits in peanuts. Arunyanark et al. (2009b) found moderate heritabilities for seed infection and aflatoxin contamination. They also found that aflatoxin traits were genetically correlated with drought tolerance traits, especially with HI, SLA and SCMR. Heritabilities of *Aspergillus* infection and aflatoxin contamination were rather low. Thus, the expected genetic gain of selection in this generation will be less for aflatoxin traits. Utomo et al. (1990) also reported that resistance to seed infection and aflatoxin production in peanut in cross AR-4 x NC 7 and GFA-2 x NC 7 are controlled by difference genes with low heritabilities (ranged from 0.20 - 0.63). Mixon (1976), however, found high heritability estimates for seed infection in cross PI 337409 x PI 331326. Percent colonization of seeds of F1 and F2 plants of reciprocal crosses between PI 337409 (resistant) and PI 331326 (susceptible) indicated low broad-sense heritability (Mixon, 1979).

PAC may be reduced with improved resistance to drought (Cole et al., 1993; Holbrook et al., 2008; 2009). Recent studies have shown a relationship of increased drought tolerance and reduced aflatoxin production (Arunyanark et al., 2009a; Girdthai et al., 2009; Holbrook et al., 2000a). However, improvement of drought resistance based on yield is also hindered by high genotype by environment (G x E) interactions (Jackson et al., 1996; Araus et al., 2002). Drought resistance traits with lower G x E interactions are promising as indirect selection tools for improving resistance to PAC.

8. Breeding for drought resistance

Peanuts are fairly drought tolerant. However, production fluctuates considerably as a result of rainfall variability. To develop drought resistance in peanut, research has been conducted to improve the performance under varying degrees of stress at various physiological stages of crop growth. Almost all modern peanut cultivars are still bred by conventional breeding methods. In the early days of peanut breeding, mass selection was commonly used to exploit natural variation within local cultivars. The pedigree method is commonly used for peanut breeding today. Genetic transformation has been successfully performed (Ozias-Akins and Gill, 2001), but no transformed cultivar has been released. Development of drought-resistant varieties by manipulating genotype variations results in higher water use efficiency. In general, the sensitivity of a given genotype to drought increases with increasing yield potential (Narasimham et al., 1977). Despite the considerable genetic resources of peanut, utilization of available genetic variability is still limited. Breeders prefer to use sources of resistance, even those with less potent resistance, that conform to market and industry standards instead of new sources with poor agronomic characteristics. Isleib and Wynne, (1992) examined the ancestral contributions of germplasm lines to modern U.S. cultivars and found that only a few germplasm lines have been used in their pedigree.

8.1 Screening technique

Screening technique for drought resistance should be used after considering the following important factors i.e. the genetic variability of drought resistance traits, the occurrence of drought in different phenological stages, the severity and duration of drought, and all potential interacting factors (Serraj, 2008). The basic procedure for evaluation of drought-resistance traits is to create drought stressed conditions and measure morphological and physiological traits (Ketring, 1984; Rucker et al., 1995; Wright and Nageswara Rao, 1994a; Robertson et al., 1980; Wright et al., 1994; Nigam et al., 2003, 2005; Nageswara Rao et al., 1988; Awal and Ikeda, 2002). A number of methods have been used in evaluating peanut genotypes in their responses to drought stress (Wright and Nageswara Rao, 1994a, b; Nautiyal et al., 2002; Holbrook and Stalker, 2003; Nigam et al., 2005). The precision or effective screening

techniques are important for breeding programs. The screening following procedures include; selection under rain-fed in drought–prone environments, line–source sprinkler irrigation, green house, rainout shelters, and hydroponic conditions.

Rain-fed in drought–prone environments: Breeder has usually screened peanut lines for drought resistance under rain-fed conditions, especially under drought–prone environments. This technique has been used in many countries (Wright and Nageswara Rao, 1994a). Number of plants, plot and experimental size are not the limitation of this technique. However, the variability in environmental conditions among sites and within test area, arising from variation in amount and distribution of rainfall and weather conditions, might confounds the intrinsic genotypic traits contributing to drought resistance.

Line–source sprinkler irrigation: Hanks et al. (1976) developed the line-source sprinkler irrigation technique, which provides a range of water application rates and therefore allows observation of plant growth and development over a range of available soil moisture conditions. This method creates a gradient of drought stress, and allows the evaluation of large numbers of genotypes at varying intensities of drought in a given environment. This system has been successfully used with corn (*Zea mays* L.) (Sorensen et al., 1980; Blad et al., 1980), sorghum (*Sorghum bicolor* (L.) Moench] (Sivakumar et al., 1981), cowpea [*Vigna unguiculata* (L.) Walp. ssp. unguiculata] (Turk et al., 1980), and peanut (Jongrungklang et al., 2008; Nageswara Rao et al., 1988, 1989). Strong wind during irrigation can influence the systematic nature of water deficits created, requiring complex statistical techniques for data analysis (Singh et al., 1991). Water pressure regulation is also an important factor controlling the uniformity of irrigation.

Greenhouse experiment: Screening of peanut lines for drought resistance has been used in greenhouse experiment (Del Rosario and Fajardo, 1988; Nautiya et al., 2002; Vorasoot et al., 2003, 2004). Plants are usually grown in pots or containers under controlled environmental conditions. This approach can solve the limitation of uncontrolled field conditions. However, the limitation of this technique is restriction the size of experimental plot. This technique can be used for studying the responses of drought resistance traits under different water regimes, not suitable for screening drought resistance in large germplasm.

Rainout shelter: Rainout shelter provides an environment which is much closer to the open field than a greenhouse experiment. This technique is designed to protect a certain area of land from the rain so that drought stress condition can be exposed to the crop on that area without the interference from any precipitations. Rainout shelter has been used to study drought tolerance peanut lines under field conditions (Branch and Kvien, 1992; Nautiyal et al., 2002). However, the main limitation of rainfall shelters is the small crop area that requires careful extrapolation of results to field areas.

Hydroponics condition: Selection among peanut genotypes for extensive root systems may be effective and valuable for improving drought tolerance (Meisner and Karnok, 1992; Songsri et al., 2008b). However, measuring root characteristics in soil medium is tedious, time consuming and labor intensive. Hydroponics culture has been used in peanut and could perhaps be useful in screening large numbers of germplasm lines or segregating populations having large root system in a breeding program to reject entries with poor root traits. However, root growth in between nutrient solution and soil medium are unlikely. Hydroponics culture has been reported as a rapid and valuable method for evaluating roots in rice (*Oryza sativa* L.) (Ekanayake et al., 1985; Price et al., 1997), wheat (*Triticum aestivum* L.) (Mian et al., 1993), sorghum (*Sorghum bicolor* [L.] Moench) (Jordan et al., 1979), cowpea (*Vigna unguiculata* [L.] Walp.) (Ogbonnaya et al., 2003) and Kentucky bluegrass (*Poa pratensis* L.) (Erusha et al., 2002), and could perhaps be useful in screening drought resistance in peanut.

8.2 Drought resistance traits

Drought resistance is a complex trait, expression of which depends on action and interaction of different morphological and physiological traits. Root systems are important plant parts for taking up water, root responses when soil moisture dries out are important mechanisms for drought avoidance (Ketring, 1984; Songsri et al., 2008b). The possession of deep and large root systems which allows access to water deep in the soil profile is considered crucially important in determining drought resistance and substantial genetic variation exists for this (Maiti et al., 2002). In peanut, substantial genotypic variation in root depth, root volume, and water extraction pattern at different depths has been reported (Ketring, 1984; Mathews

et al., 1988; Wright et al., 1991; Chapman et al., 1993b). The ability to partition dry matter into harvestable yields under limited water supply is an important trait for drought-resistant genotypes (Chapman et al., 1993a, c; Nigam et al., 2005). Harvest index or partitioning efficiency is an important trait that provides a measure of total biomass actually partitioned into pod yield. It has also been identified as a drought resistance mechanism in peanut (Nigam et al., 2003, 2005). Duncan et al. (1978) suggested that partitioning of assimilates expressed as HI has considerable effects on pod yield, and breeding for high pod yield might be accomplished by selection for high HI.

Identification of simple to observe morpho-physiological and phenological traits, drought-adaptive mechanisms and processes that confer drought resistance is a priority activity in drought research. Direct selection for pod yield and/or biomass under stressed conditions can be used to screen drought-resistant genotypes. However, the limitations of this approach are high resource investment and poor repeatability of the results due to the large G x E interaction that results in slow breeding progress (Wright et al., 1996). More rapid progress may be achieved by using physiological and morphological traits (Nigam et al., 2005).

Several physiological and morphological traits have been associated with drought stress adaptation. Passioura (1986) have proposed a simple model of yield based on the facts that pod yield is a function of T, water use efficiency (WUE), and HI. Drought tolerance might be enhanced by improving soil water extraction capability or improvements in WUE, or integration of both (Wright and Nageswara Rao 1994a, Hebbar et al. 1994). Wright et al. (1994) demonstrated genetic differences in peanut for TE or WUE which is defined as gram of dry matter produced per kilogram of water transpired. Improvement of WUE could potentially lead to increased yield under limited moisture availability. However, WUE is not easy to measure and may not be a feasible selection criterion in large segregating breeding populations.

Wallace et al. (1993) suggested that indirect selection for yield will be most effective when applied to traits that already integrate most of the genetic and environmental effects that lead to yield. Farquhar et al. (1982) proposed that the transpiration efficiency of a genotype could be estimated by measuring the carbon isotope discrimination (Δ) in leaves. Wright et al. (1988) and Wright et al. (1994) have also found WUE to be negatively correlated with Δ and SLA over wide ranges of varieties and environments, but analysis of Δ are expensive and not feasible everywhere. SLA which is negatively related to leaf thickness and photosynthetic capacity can be measured easily and inexpensively. Although SLA is affected by environment and genotype, the relationship between SLA and Δ is apparently stable across environments in peanut (Nageswara Rao and Wright 1994). SLA can be easily and inexpensively measured, and it is being used in a large–scale screening programmes for improved drought resistance in Australia and India (Wright and Nageswara Rao, 1994b). However, care must be taken when using specific leaf area as a selection criterion since it is significantly influenced by time of sampling and leaf age (Wright and Hammer, 1994; Nageswara Rao et al., 1995).

The hand–held portable soil plant analysis development (SPAD) chlorophyll meter was used for rapidly assessing drought tolerance in peanut (Nageswara Rao et al., 2001; Arunyanark et al., 2008; Nigam and Aruna, 2008). The SPAD chlorophyll meter reading (SCMR) is an indicator of the photo–synthetically active light–transmittance characteristics of the leaf, which is dependent on the unit amount of chlorophyll per unit leaf area (chlorophyll density) (Richardson et al., 2002). In peanut, significant and positive correlations between SCMR and chlorophyll content and chlorophyll density (Akkasaeng et al., 2003; Arunyanark et al., 2008) have been reported. Leaf photosynthesis is generally correlated with chlorophyll content per unit leaf area, and hence the SPAD chlorophyll meter can provide a useful tool to screen for genotypic variation in potential photosynthetic capacity (Nageswara Rao et al., 2001). The relationship between TE and SCMR in peanut was positively correlated (Bindu Madhava et al., 2003; Sheshshayee et al., 2006).

Nageswara Rao et al. (2001) and Upadyaya (2005) found a significant negative correlation between SCMR and SLA, and Nageswara Rao and Wright (1994) found these associations relatively stable across environments suggesting that this chlorophyll meter could be used as a rapid and reliable measure to identify genotypes with low SLA and hence high TE in peanut. Genotypic correlations between HI and SLA and SCMR were also found in peanut under well-watered and drought conditions (Songsri et al. 2008c).

8.3 Inheritance of drought resistance

Hubick et al. (1988) reported that heritability estimates using reciprocal crosses of Tifton–8 and Chico were high for TE and especially for Δ, and there was no significant G x E interaction for Δ in peanuts. Songsri et al. (2008c) found that heritabilities of physiological traits for drought resistance in peanut were high (h^2 > 0.50) under drought and well-watered conditions, and physiological traits such as SLA, SCMR, HI, and drought tolerance index of pod yield and biomass were genetically associated well with agronomic traits under long periods of drought. Ntare and Williams (1998) also reported that heritability of pod yield was lower than partitioning coefficient but higher than other physiological components (crop growth rate and duration of reproduction growth) of their yield model. Cruickshank et al. (2004) also found that heritability estimates for HI were high (varied from 58-85 %) and varied significantly among crosses depending on levels of genetic variation in parents. Heritability (h^2) estimates in early generations (F_3 and F_4) of T, TE and HI have been reported. Broad–sense heritability estimates of T (H^2 ranging from 0.00 to 0.70), TE (H^2 ranging from 0.49 to 0.86) and HI (H^2 ranging from 0.16 to 0.85) varied among peanut crosses and traits depending on levels of genetic variation in parents (Cruickshank et al., 2004).

Carbon isotope discrimination had a negative correlation with transpiration efficiency and top dry weight (–0.78 and –0.48, respectively) and had a positive phenotypic correlation with harvest index and seed yield (0.63 and 0.64, respectively) and also had a high correlation with water use efficiency (r ranging from –0.88 to –0.92) (Hubick et al., 1986, 1988; Wright et al., 1988, 1994). Additive gene action has been the main factor responsible for variation in many agronomic traits in peanut.

Previous studies reported that HI and SLA are mainly under additive genetic control and SCMR was found to be under the influence of both additive and non-additive gene effects (Dwivedi et al.1998, Jayalakshmi et al. 1999, Lal et al. 2006, Nigam et al. 2001, and Suriharn et al. 2005). Hence, selection should be effective. Nigam et al. (2001) found that the selection for SLA and HI can be effective in early generations. They also suggest that the selection can be done in late generation to exploit the effect of additive x additive interaction.