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Developing Drought Tolerance in Soybean Using Physiological Approaches

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ABSTRACT

In recent years soybean has become a predominant rainy season oilseed crop under rainfed agro-ecosystem in India. However, the average productivity of crop continues to be very low as compared to world average as well as its climatic potential in India. Occurrence of drought at one or the other stages of crop growth is attributed as one of the major factors responsible for low productivity of soybean in India. Therefore, in order to improve the productivity and sustainability of soybean in India, there is an urgent need to make systematic efforts to develop genotypes which are better adapted to drought conditions. Physiological dissection of complex traits like drought is a first step to understand the genetic control of tolerance. Identification of physiological traits and genetic sources possessing such traits will ultimately enhance the efficiency of conventional as well as molecular breeding strategies for drought tolerance. The current review highlights the efforts needed to characterize the soybean growing environment in India and need to exploit germplasm in developing drought tolerance in soybean using physiological and biochemical traits. Also, the review analyses the present gaps in research and required focus to fill these gaps in order to utilize these traits for developing future soybean plant which is tolerant to drought conditions.

Key words: Breeding, drought, physiological traits, soybean, India

Among modern agricultural commodities, soybean [*Glycine max* (L.) Merrill] has a prominent place as the world's most important seed legume, which contributes 25 per cent to the global vegetable oil production, about two thirds of the world's protein concentrate for livestock feeding and is a valuable ingredient in formulated feeds for poultry and fish. Currently the crop is grown in 103 million ha globally with an annual production of 261 million tons and average productivity of 2 533 kg per ha (FAOSTAT, 2013). India ranks fourth in terms of area and fifth in production of soybean in the world.

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The commercial cultivation of soybean in India commenced in late sixties; thereafter it has made an unprecedented phenomenal growth, having no parallel in the crop history world over (Bhatia *et al*., 2011). Starting from an area of just 30, 000 ha in 1970, soybean has reached to 10.69 million ha in 2012 (SOPA, 2013). The production and productivity levels of 14 000 tons and 0.43 t per ha in 1970 have increased to 14.14 million tons and 1.2 t per ha in 2012, respectively (DAC, 2013). Although, the contribution of India in the world soybean area is 10 per cent, it is only 4 per cent in total world's production indicating its relatively lower productivity as compared to World average.

At present the area under soybean is mainly spread in latitudinal belt of about 15 to 25^0 N comprising the states of Madhya Pradesh, Maharashtra, Rajasthan, Chhattisgarh, Andhra Pradesh and Karnataka (Bhatia *et al*., 2008). Besides improving the socio-economic conditions of small and marginal farmers of the country, the crop contributes 25 per cent of the total edible oil produced in the country and earns substantial amount of foreign exchange (INR 70 000 million) by exporting defatted oil cake (DOC) (Paroda, 1999; Bhatia *et al*., 2011). As soybean is the cheapest source of high quality protein, the crop has potential to alleviate large scale protein malnutrition in the country.

Despite the spectacular growth in area and production, the average productivity of the crop (1.2 t/ha) in India is less than half the world average (2.53 t/ha) and one third of its climatic potential (3.5 t per ha) (Bhatia *et al*., 2008). Several abiotic, biotic and socio-economic factors, responsible for low productivity of soybean in India have been identified (Paroda, 1999; Joshi and Bhatia, 2003). Due to rainfed nature, occurrence of severe drought conditions at one or the other stages of crop growth and development is the most important factor limiting soybean productivity in India (Joshi and Bhatia, 2003). The current climate in terms of drought and temperature are already affecting the productivity of soybean and the problem is expected to further accentuate in future (IPCC, 2007). As the livelihood of millions of small and marginal farmers depends on this crop and looking at its importance in country's oil economy, there is an urgent need to improve soybean productivity, which can be achieved mainly by improving the resistance/tolerance to drought.

Barring a few efforts, no systematic studies have been taken up to develop drought tolerant soybean varieties in India in the past. The lack of such efforts is attributed to non-availability of proper screening facilities, lacking clear knowledge on nature of drought and its impact on soybean and most importantly poor understanding of physiological and biochemical responses of Indian soybean varieties to drought occurring with varying intensities at different stages of growth. The following review highlights the efforts needed to developing drought tolerance in soybean using physiological and biochemical traits. Also the review analyses the present gaps in research and required focus to fill these gaps in order to utilize these traits for developing future soybean plant which is tolerant to drought conditions.

Characterization of soybean growing environments

Progress in plant improvement for yield relies on the identification of genotypes better adapted to their environment. Therefore, to maximize the impact of using specific traits, breeding strategies require a detailed knowledge of the environment where the crop is grown, genotype x environment interactions and fine-tuning the genotypes suited for local environments. In India, soybean is mainly grown on Vertisols and associated soils which have good water holding capacity. The average rainfall in major crop growing region is 900 mm which varies greatly across locations (400 to more than 1200 mm) and seasons (Bhatia *et al*., 2008). Occurrence of long dry spells at one or the other stages of crop growth is a common phenomenon and occurrence of terminal drought due to early withdrawal of monsoon is the most critical phenomenon affecting crop yields once in 2-3 years in major soybean growing regions of India. Looking at the rainfall variability, there is an urgent need to characterize the crop environment so that the region-wise patterns of drought and its impact on soybean growth and yield are quantified and appropriate breeding strategies could be worked out. This would also help in identification of contrasting sites for multi-location testing for genotypes and crossing material for drought tolerance. In this regard, the crop models could be of great help as they capture feedback between plant growth and soil water depletion and have been shown to characterize water-limited environments better when compared with standard indices computed from climatic data (Muchow *et al*., 1996; Chenu *et al*., 2011).

Genetic resources

Trait introgression into a single genetic background either through conventional or molecular breeding requires identification of suitable trait donor parental lines, with a robust marker-assisted selection (MAS) strategy. Hence, identification of such donor parents is essential, as well as the development of genomic resources for soybean. Therefore, plant genetic resources are the most valuable among all of the natural resources. Currently, the total soybean germplasm collection in India is estimated to be about 7 500, which includes about 4 100 accessions at National Repository (Directorate of Soybean Research) and rest scattered at different centres of AICRP on soybean and also at NBPGR, New Delhi. There is an urgent need to consolidate the total holding at one place and eliminate duplicity in available soybean germplasm in India. Also, the available number of germplasm accessions is meager as compared to more than 23 000 accessions with China where soybeans originated (Xie *et al*., 2005) and USDA (GRIN, 2013). The USDA collection includes 21 000 accessions of *Glycine max* and rests are of 20 different wild species of Glycine. Therefore, there is an urgent need to enrich our germplasm pool through these sources and other important national collections such as Japan, South Korea, Russia and even AVRDC could also be a potential source for soybean germplasm.

Even with existing collection, there is an inadequate use of germplasm in soybean breeding programmes. Out of 100 soybean varieties released so far in India, 25 per cent are either direct introduction from USA (Davis, Clark 63, Lee, Hardee, Improved pelican Bragg, and Monetta), or indigenous or exotic material (Kalitur, Type 49, Punjab 1, JS 2, ADT 1, Shilajeet, KM 1, Co 1, and Gujrat Soya 2, NRC 7). Rest 75 varieties which have been bred in India, their parentage are mostly these introduced cultivars except about 10 odd varieties where exotic or indigenous germplasm is used as one of the parent (Agrawal *et al.,* 2010). The main reason for the limited use of germplasm in soybean improvement programmes is the lack of information on a large number of the accessions, particularly for traits of economic importance, which display a great deal of genotype-by-environment interaction (GEI). Therefore, there is a need to thoroughly characterize the existing germplasm for economically important traits including drought and develop a core (Frankel, 1984) and mini-core collections (Upadhyaya and Ortiz, 2001; Upadhyaya *et al*., 2006), which represent the true genetic variability in available germplasm and is generously distributed to breeders for enhanced germplasm use in breeding programme and to other interested soybean researchers in the country.

Importance of physiological traits in drought tolerance

Among the various abiotic stresses that curtail crop productivity, drought is the most recalcitrant to breeding (Tuberosa and Salvi, 2006), because plants use various mechanisms to cope with drought stress. In the past, drought tolerance breeding has been hindered by the quantitative inheritance of the trait and our poor understanding of the physiological basis of yield in water-limited conditions (Sinclair, 2011) as well as by limitations in technology for systematic phenotyping. The physiological dissection of complex traits like drought is a first step to understand the genetic control of tolerance and will ultimately enhance the efficiency of molecular breeding strategies. A physiological approach has an advantage over empirical breeding for yield *per se*, because it increases the probability of crosses resulting in additive gene action for stress adaptation, provided that the germplasm is characterized more thoroughly than for yield alone (Reynolds and Trethowan, 2007).

The use of physiological traits (PTs) in a breeding programme, either by direct selection or through a surrogate such as molecular markers, depends on their relative genetic correlation with yield, extent of genetic variation, heritability, and genotype x environment interactions. It is important to note that using specific traits, breeding strategies are effective only when these traits are properly defined in terms of the stage of crop development, at which they are relevant, the specific attributes of the target environment for which they are adaptive and their potential contribution to yield

(Reynolds and Trethowan, 2007). When significant genetic diversity for a physiological trait in a germplasm collection for the given species is established, it is imperative that the relevance of the trait as a selection criterion be determined. Identification of drought-adaptive PTs and mechanisms is time consuming and costly; however, if successful, the benefits are likely to be substantial.

Conceptual framework for drought adaptation in soybean

There are basically two frameworks which identify character-istics for improved drought resistance in crop plants including soybean (Turner *et al.,* 2001). The first framework also called as "Drought-Resistance Framework", relies on specific physiological and biochemical characteristics that lead to improvement under drought conditions through drought escape, drought avoidance through dehydration postponement, and drought tolerance. Drought escape allows the plant to complete its life cycle during the period of sufficient water supply before the onset of drought. The second mechanism, drought avoidance, involves strategies, which help the plant maintain high water status during periods of stress, either by efficient water absorption from roots or by reducing evapo-transpiration from aerial parts. The third mechanism, drought tolerance, allows the plant to maintain turgor and continue metabolism even at low water potential using mechanisms such as protoplasmic tolerance, synthesis of osmoprotectants, osmolytes or compatible solutes (Nguyen *et al*., 1997).

The second framework also termed as "Yield Component Framework" which mainly takes into account yield variation in terms of traits affecting water use (WU), water use efficiency (WUE) and harvest index (HI) (Turner *et al*., 2001) and is represented by equation Y = WU x WUE x HI (Passioura, 1977). Deep rooting, osmotic adjustment and early vigour leading to early ground cover are the traits associated with WU. WUE on a whole plant basis (WUE_{plant}) is defined as a ratio of total dry matter or yield to water use and at level of leaf (WUE_{Leaf}) is a ratio of CO_2 assimilation into the photosynthetic biochemistry (A) to water lost *via* transpirations through stomata (T). It is basically a function of stomatal regulation for transpiration and CO_2 intake and photosynthetic efficiency. WU and WUE mainly contribute to greater yield through increase in total biomass, which must then be converted to yield *via* a higher HI. Traits like sensitivity of reproductive processes and translocation of reserves to grain could influence HI index under drought conditions. Considering the overall contributions of these three yield drivers, optimum transpiration is the most important for improving the yield potential (biomass) in drought environments, while stable HI is associated with higher yield potential (Blum, 2009; Salekdeh *et al*., 2009).

Both the frameworks need to be considered if progress is to be made in breeding for drought tolerance using physiological approaches in soybean.

Manavalan *et al*. (2009) suggested that in soybean, maintenance of optimum transpiration, leading to increased WUE, is one of the strategies to improve yield. Nine secondary traits have been reported to be associated with the likelihood of increasing or maintaining T during drought. These traits are phenology, photoperiod sensitivity, developmental plasticity, leaf area maintenance, heat tolerance, osmotic adjustment, early vigour, rooting depth and rooting density. Transpiration efficiency and leaf reflectance are the other two traits associated with WUE (Purcell and Specht, 2004). Some other traits associated with WUE include early vigour, photo-protective mechanisms including antioxidant systems, regulation of water flow *via* aquaporins and signaling molecules such as abscisic acid (ABA) (Reynolds, 2002; Reynolds and Tuberosa, 2008). Similarly, for HI, the extreme sensitivity of reproductive processes to drought may result in the reproductive failure, which is associated with low HI, and may eliminate benefits associated with favourable transpiration or WUE. Considering the two above frameworks for drought resistance, it is crucial to target specific physiological mechanisms and to identify those traits most relevant to the improvement of drought tolerance in soybean under Indian conditions.

Physiological traits

Phenology: Phenology is indeed an important component in the adaptation of plants to any given environment. The early escape from progressively intensifying moisture stress, through the manipulation of plant phenology, is the most commonly exploited genetic strategy used to ensure relatively stable yields under terminal drought conditions (Richards, 1991). Shorter life cycle and short growth duration can decrease the total water use by plants, compared with longer duration and larger leaves because of decreased transpiration. Several studies (Manavalan *et al.,* 2009) have shown that short-duration cultivars escape drought because they complete their life cycle before the occurrence of drought, whereas long-duration cultivars have greater chances of being exposed to severe drought or heat stress, particularly, during the later stages of crop development.

However, the degree of yield loss due to soil moisture deficits depends on the phenological timing of the stress (Schou *et al*., 1978). Under rainfed environment, identification of matching phenology to available resources is the key to optimize productivity of given crop (Ishibashi *et al*., 2003). Occurrence of terminal drought due to early withdrawal of monsoon is the major factor that limits the duration of the crop and its productivity in major soybean growing region of India (Joshi and Bhatia, 2003; Bhatia and Ramesh, 2009) Therefore, poor yields obtained under rainfed agro-ecosystem of central India may be improved by selecting genotypes, which have better matching crop development to periods of sufficient moisture. The studies carried out by Bhatia and Ramesh (2009) indicated a significant curvilinear association of soybean yield with days to flowering, days to maturity and seed fill duration under central Indian conditions which is the epicenter of soybean cultivation. They reported that soybean genotypes which flower in about 37 days, mature in about 90 days and have a seed fill duration of about 38 days will utilize the available soil moisture efficiently and give maximum yield. As the genetics of earliness are well known and there is sufficient variability in the phenology of Indian soybean varieties (Bhatia *et al*., 2003) and available germplasm, this trait needs to be exploited in breeding programmes aimed at developing drought adapted varieties in India.

It is also important to consider that the occurrence of drought is very unpredictable under semi-arid and dry regions, therefore, if drought occurs during the sensitive stages of these short life-cycle cultivars, the impact could be very severe. Soybean plants, which are subjected to stress during flower formation, have shorter flowering periods (Sionit and Kramer, 1977) while water stress during later phases of soybean reproductive development has been reported to accelerate senescence, in turn decreasing the duration of the seed-filling period (Korte *et al*., 1983; Meckel *et al*., 1984). Therefore, in addition to phenology *per se* (mean time to a phenological stage), plasticity of phenological development merits consideration as a distinct trait influencing crop adaptation.

Early vigour: The weather conditions during early crop duration in India are more or less characterized by high humidity and low solar radiation. These conditions lead to poor evapo-transpiration from the crop canopy. Early and vigorous establishment under conditions of low evapotranspiration may allow rainfed crops to optimize WUE and limit the loss of water due to direct evaporation from the soil surface. This leaves more stored water available for later developmental stages when soil moisture becomes progressively exhausted and increasingly limiting for yield (Richards *et al*., 2002; Slafer *et al*., 2005; Richards, 2006; Rebetzke *et al*., 2007; Reynolds and Tuberosa, 2008). Early establishment also reduces the occurrence of inhibition of stomatal conductance as a consequence of root-borne signaling such as from ABA through the xylem flow (Davies *et al*., 2000; Ren *et al*., 2007), caused by an excessively shallow and superficial root system (Blum, 1996; Giuliani *et al*., 2005). As a trade-off, excessively vigorous canopy development may cause early depletion of soil moisture. The optimal degree of vigour will thus depend on the environments in which cultivars to be grown within the geographical area targeted by a breeding programme also known as the target population of environment (TPE) (Fischer *et al*., 2003). Early vigour has been exploited to improve WUE and yield in many crops like wheat (Asseng *et al*., 2003; Richards, 2006; Rebetzke *et al*., 2007). Incorporation of early vigour trait which is an integration of several physiological factors like germination, water uptake, good root and shoot development, and leaf area will benefit the soybean breeding programme that is targeted for stress prone areas.

Therefore, a critical evaluation of these responses to moisture stress may provide valuable information about drought tolerance of various genotypes.

Root-related traits

Root morphology and plasticity: Soybean plants often undergo substantial water deficits, even though water is readily available only slightly deeper in the soil layer. Significant correlations exist in soybean between drought resistance and various root traits such as dry weight, total length, volume, and number of lateral roots (Read and Bartlett, 1972; Hoogenboom *et al.*, 1987; Hirasawa *et al.,* 1994; Liu *et al*., 2005). Plants can adapt to drought by developing a longer taproot which helps reach the lower soil layers where water is available (Taylor *et al*., 1978). In soybean, cultivars with larger root length densities and deeper rooting systems have been reported to be more tolerant to drought stress conditions (Sponchiado *et al*., 1989; Sloane *et al*., 1990; Hudak and Patterson, 1996). One of the major factors influencing soybean rooting depth is taproot elongation rate. As taproots are the first formed roots in soybean, identification of genotypes with rapidly elongating taproots under non-stress conditions may allow the determination of deeper rooting ability (Kaspar *et al*., 1984). It has been established that if a plant develops a large root system during its early vegetative growth, it would be in an excellent position to maintain turgor under drought conditions (Hirasawa *et al*., 1994). Overall, root traits show strong potential for improvement of drought resistance through breeding.

The main drawback to the study of root features and their use as selection criteria relates to the difficulty of phenotyping field-grown plants (Richards, 2006). A number of techniques such as exaction and coring methods have been used to estimate root mass and distribution (Nissen *et al.,* 2008). Also some recently developed non-destructive techniques such as root imaging through minirhizotron tubes allows quantification of root growth over time. Though time consuming and laborious, understanding the physiological mechanisms and genetic regulation of root adaptation to drought will not only be rewarding in conventional breeding but also help to identify specific genes and metabolic pathways for gene based marker selection with better root related traits.

There are some other indirect traits which can be used as surrogate measurement for root growth. These traits include lower canopy temperature and increased stomatal conductance which are indicative of increased water use under drought conditions (Manavalan *et al.,* 2009). Canopy temperature can be measured by an infrared thermometer (IRT) which is a simple, fast and inexpensive device. Similarly, stomatal conductance can be measured using leaf porometer or portable photosynthesis systems.

Nitrogen fixation and metabolism under drought: Nitrogen (N_2) fixation in legumes is very sensitive to soil drying. Under drought conditions, soybean not only suffers losses of CO_2 accumulation and reduced leaf area development, but its symbiotic N_2 fixation is also especially vulnerable (Sinclair and Serraj, 1995). In dry soils, this results in a reduced supply of N_2 for protein production, which is the critical seed product of the plant, and consequently lower crop yields (Purcell and King, 1996). Several factors have been related to the inhibition of N_2 fixation under drought, including reduced oxygen availability, reduction in carbon flux to nodules, decline in nodule sucrose synthase activity, increase in ureides and free amino acids (Durand *et al*., 1987; Gonzalez *et al*., 1995; Arrese-Igor *et al*., 1999; King and Purcell, 2005). Soil drying also leads to the accumulation of ureides in soybean leaves and is thought to be an inhibitor of nodulation (Sinclair and Serraj, 1995). Substantial genetic variation was found in the sensitivity of N_2 fixation in response to soil drying (Sall and Sinclair, 1991). Screening for petiole ureide levels is an effective initial approach to identify soybean lines whose N_2 fixation is more tolerant of soil drying.

Shoot-related traits

Osmotic adjustment: Osmotic adjustment (OA) is a metabolic process resulting in a net increase in intercellular solutes in response to water stress. Under decreased soil moisture conditions, osmotic adjustment helps in maintenance of turgor and hence the integrity of metabolic factions. OA has been reported in legumes with a high tolerance to water stress (Ford 1984, Ashraf and Iram, 2005). In soybean, Silvente *et al*. (2012) have demonstrated critical difference in physiological responses in drought tolerant and susceptible genotypes and have identified the metabolic pathways that are affected by short-term water limitation in soybean plants. OA has been shown to maintain stomatal conductance and photosynthesis at lower water potentials, delayed leaf senescence and death, reduced flower abortion, improved root growth and increased water extraction from the soil as water deficit develops (Turner *et al*., 2001). Among different types of organic and inorganic solutes which accumulate in the cytosol to lower osmotic potential, proline is the most widely studied because of its considerable importance in the stress tolerance. Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. Thus, OA has implication in sustaining yield under water deficit conditions in soybean.

Water use efficiency: Water use efficiency in one of the physiological traits that has been associated with drought tolerance in soybean. Genetic variation in WUE has been reported in recent years for many field crops (Ashok *et al*., 1999; Sheshshayee *et al*., 2003) including soybean (Mian *et al*., 1996). High WUE can contribute to crop productivity under drought and low genotype x environment interaction and a moderate to high broad-sense heritability for this trait (Ashok *et al*., 1999; Condon *et al*., 2004) suggest that breeding efforts could be attempted for this trait. The positive association between WUE and total biomass yield in a drought environment suggests that improvement of the WUE of a crop plant should result in superior yield performance if a high harvest index can be maintained (Wright, 1996). It has been proposed that the aperture of stomata could be regulated in such a way that a partial closure of stomata at a certain level of soil water deficit might lead to an increase in WUE (Liu *et al*., 2005). Therefore, a deep understanding of the physiological basis for stomatal regulation and improved WUE in drought-stressed plants is required (Liu *et al*., 2005). Another relevant trait related to WUE is transpiration efficiency (TE), which is the assimilation or dry matter accumulation per unit of transpiration (Fischer, 1981). TE is under genetic control and excludes amount of water lost by soil evaporation, and hence should be considered as a potential trait.

Several methods have been suggested for measuring WUE in crop plants. It can be determined at the whole plant level by gravimetric, at single leaf level by gas exchange methods and using carbon isotope discrimination. Gravimetric approach involves the accurate determination of the total water used by a plant over a specific period of crop growth and the total biomass accumulated by the plant over the same period. This is achieved by accurate determination of water content in the soil by weighing the containers at regular interval (Udaykumar *et al*., 1998). Gas exchange approach for determining water use efficiency relies on the fact that at single leaf level, WUE is the ratio of carbon assimilation (A) to transpiration rate (T). A portable infrared gas analyzer (IRGA) is routinely used to determine gas exchange traits such as carbon assimilation rate, stomatal conductance, transpiration rate and intercellular CO_2 concentration, which can give fairly good information on leaf level WUE in soybean genotypes. However, the fact that carbon isotope discrimination ($\Delta^{13}C$) is a powerful surrogate for WUE, has made it possible to rapidly assess the genetic variability in WUE (Farquhar and Richards, 1984; Farquhar *et al,* 1989). The determination of stable isotope ratio requires mass spectrometer and such facilities in India are available at Crop Physiology Division, UAS Bangalore, which must be tapped for soybean programme aimed at improving drought tolerance.

Alternative approaches to measure WUE: Before quantifying the stable carbon isotope ratios using mass spectroscopy, large number of genotypes could be screened using simpler alternative traits such as specific leaf area, specific leaf nitrogen, leaf chlorophyll content and wax content, which have also been shown to be associated with WUE.

Specific leaf Area (SLA): SLA is defined as the ratio of leaf area to its dry weight and is indirect measure of leaf expansion. A positive correlation between SLA and $\Delta^{13}C$ has been demonstrated in groundnut (Rao and Wright, 1994) indicating that SLA is negatively associated with WUE. These associations suggest that for rapid assessment of genotypic variability in WUE, SLA can be used as an alternative.

The specific leaf nitrogen: WUE is basically a function of stomatal regulation for transpiration and CO_2 intake and photosynthetic efficiency. The photosynthetic efficiency largely depends on the biochemical efficiency of carbon fixation in chloroplast. As the 80 per cent of leaf protein is RuBP enzyme, measuring leaf nitrogen and, hence, leaf protein content is an important indicator of photosynthesis. SPAD chlorophyll meter readings have been well correlated with nitrogen content of the leaf (Rao *et al*., 2001) and therefore, simple measurements using SPAD chlorophyll meter could provide fairly good indication in genotypic variability in this traits.

Chlorophyll contents: Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Both the chlorophyll a and b are prone to soil dehydration (Farooq *et al*., 2009). Decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought (Kypoarissis *et al*., 1995; Zhang and Kirkham, 1996). Loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis.

In recent years, availability of simple hand-held instruments has made it possible to measure chlorophyll content in large number of genotypes with ease. One such instrument is SPAD chlorophyll meter (Minolta Corp., Ramsey, New Jersey, USA) that measures unit less value also referred to as the SPAD chlorophyll meter reading (SCMR) which are directly related to chlorophyll content in leaves. The SCMR have also been shown to strongly correlate with SLA and SLN (Rao *et al*., 2001). Thus SCMR can be used as a simple alternative technique to estimate WUE at least as an initial screening.

Chlorophyll fluorescence: In fact, in recent years, fluorescence measurement of chlorophyll a have been used as a powerful tool to understand, study and quantify the non-stomatal (photochemical) inhibition to photosynthetic efficiency and screening of tolerant genotypes under water stress conditions (Ohashi *et al*., 2006; Rahbaria *et al*., 2011). The principle underlying chlorophyll fluorescence is based on the fact that the light energy absorbed by chlorophyll molecule in a leaf can undergo one of the three fates: it can be used to drive photosynthesis (photochemical), extra energy can be dissipated as heat or it can be re-emitted as light *i.e.* chlorophyll fluorescence. By measuring the yield of chlorophyll fluorescence, information on changes in the efficiency of photochemistry and heat dissipation can be obtained. Hence, the measurement of various parameters of chlorophyll fluorescence can be used as reliable indicators to evaluate the energetic/metabolic imbalance of photosynthesis and yield performance across genotypes under water deficit (Araus *et al*., 1998, Rathod *et al.,* 2011). The important parameters of leaf fluorescence measurements are variable fluorescence (Fv) which is the difference between maximum and minimal fluorescence (Fm-Fo). The variable to maximum fluorescence ratio (Fv/Fm) is an indicative of potential or maximum quantum yield of PS II. The declining slope of Fv/Fm is a good indicator to evaluate photo-inhibition of plants exposed to environmental stresses such as drought and heat, accompanied by high irradiance. According to Paknejad *et al*. (2009), drought stress reduces the variable (Fv) and initial (F_0) fluorescence parameters and quantum yield (Fv/Fm). The study of chlorophyll fluorescence parameters is a simple, non-destructive method, rapidly leads to valuable results. The availability of fast, less expensive, hand held and portable fluorimeters, which measure various parameters of fluorescence has now made easy to carry out such measurements under field conditions for screening of large number of genotypes.

Wax content and leaf pubescence density: The effectiveness of stomatal control over transpiration efficiency and hence, WUE, would increase if non-stomatal transpiration pathways are eliminated. An important non-stomatal water vapor pathway is the leaf cuticle. Cuticular transpiration is affected by the amount, composition, and physical configuration of epicuticular wax deposits (Blum, 1975; Schonherr, 1976). Genetic variation in epicuticular wax content or cuticular transpiration is found in many crops including soybean (Paje *et al*., 1988; Kim *et al*., 2007). Heavy deposition of epicuticular wax also affects the spectral characteristics of leaves (Blum, 1975) to the extent that net radiation (the energy load on the leaf) is reduced. Relatively rapid colorimetric (Ebercon *et al*., 1977) methods for measurement of epicuticular wax content are available for screening large number of genotypes.

Leaf pubescence density (PD) is an important component for the adaptation of soybean to drought-prone environment (Du *et al*., 2009). Pubescence density in soybean can reduce leaf temperature, restrict loss of water by transpiration, enhance photosynthesis, increase vegetative vigour, greater root density, and a deeper root extension and increased WUE (Clawson et al., 1986; Yi *et al*., 1986, Du *et al*., 2009). The pubescence density can be measured by simple microscopic observations and hence needs attention as a useful trait in soybean,

Other intrinsic tolerance mechanisms at cellular level: Many other physiological and biochemical mechanisms provide intrinsic tolerance to drought at the cellular level and genotypic differences could be studied. This includes the production of reactive oxygen species (ROS) and their elimination by antioxidants and antioxidant enzyme system. Drought induces oxidative stress in plants by generation of reactive oxygen species (ROS) (Farooq *et al*., 2009). The ROS such as O_2^-, H_2O_2 and $OH^•$ radicals, can directly attack membrane lipids and increase oxidative damage to proteins and DNA (Mittler, 2002).

To minimize the deleterious effects of oxidative stress, plants have evolved a complex enzymatic and non-enzymatic antioxidant system, such as low-molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS scavenging enzymes (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) (Apel and Hirt, 2004).). However, the balance between ROS production and levels of antixodants in combination with activities of antioxidative enzymes determines whether oxidative damage will occur (Moller *et al*., 2007). In genotypes with weak antioxidant defense system may not be able to remove the excessive ROS produced during water stress which may lead to loss of membrane integrity, and damage to protein and DNA in the cells. Drought-induced overproduction of ROS increases the content of malondialdehyde (MDA). The content of MDA has been considered an indicator of oxidative damage (Moller *et al*., 2007). MDA is considered as a suitable marker for membrane lipid peroxidation. Alternatively, the rate of injury to cell membrane by water stress can also be estimated by measuring electrolyte leakage from the cell. The method is based on desiccation *in vitro* of leaf tissues by polyethylene glycol (PEG) and subsequent measurement of electrolyte leakage into a deionized aqueous medium (Sullivan, 1971). The amount of electrolyte leakage, evaluated by electrical conductivity measurement, is a function of cell membrane stability (CMS). A decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS. This technique has been used in many crops (Blum and Ebercon, 1981; Premachandra and Shmada, 1987) including soybean (Krishnamani *et al*., 1984). All these studies conclude that the degree of CMS was well correlated with tolerance of genotypes to water stress conditions.

Being a rainfed crop, drought is a major limiting factor to soybean productivity in India and hence a systematic national programme to develop adaptive soybean genotypes is needed. In order to do so, there is an overwhelming consensus that there is a need to properly characterize the soybean growing environment and on need to properly understand the physiological and biochemical processes associated with drought in soybean. Identification of physiological and biochemical traits, evaluation of large number of germplasm and identification of genetic sources possessing such traits can only lead to breeding soybean varieties for drought tolerance based on such specific traits. Identification of QTLs and molecular markers can further hasten the development of drought tolerant varieties using molecular assisted selections in breeding programmes. Focus has to be on the traits which could substantially contribute to improving transpiration and WUE in water limited environments. These traits include osmotic adjustment capacity, phenological plasticity, root traits, epicuticular wax, canopy temperature, stomatal response, disruption to photosynthetic capacity, disruption of PS I and PS II, production of ROS and their elimination by antioxidants and antioxidant enzyme systems and membrane integrity. Understanding of physiological and biochemical processes, identification of traits and genetic sources for drought tolerance would go a long way in developing soybean varieties better adapted to current and future climatic conditions of the country.

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Genetic Variability, Association and Path Analyses in Advanced Generation Fixed Lines of Soybean [*Glycine max* (L.) Merrill]

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ABSTRACT

The present study was carried out during kharif *2012 considering twenty six advanced generation genotypes of soybean. Different genetic parameters namely, genetic variability, heritability, genetic advance, correlation and path analyses were studied. Number of branches per plant, number of pods per plant, number of seeds per plant, biological yield per plant and yield per plant showed high heritability and high genetic advance whereas, high heritability with moderate genetic advance was found for plant height and 100 seed weight indicating the preponderance of additive gene action. Seed yield per plant exhibited positive and highly significant correlation with biological yield, number pods per plant, number of seeds per plant, plant height, harvest index, vegetative phase and number of seeds per pod. Phenotypic and genotypic path analyses of different traits revealed that number of pods per plant, biological yield per plant number of seeds per plant and harvest index depicted substantial phenotypic and genotypic direct effect on seed yield. These traits have also shown positive indirect effect* via *each other which indicated that simultaneous improvement of these traits and ultimately the yield can be achieved.*

Key words: Advanced generation, correlation coefficient, path analysis, soybean

Soybean [*Glycine max* (L.) Merrill], a self pollinated diploid (2n = 2x = 40) food legume belonging to family Leguminosae syn. Fabaceae, subfamily Papilionoideae originated in is north and central China. Soybean ranks first amongst oilseed crops in the world as well as in India. India ranks fourth in terms of soybean area in the world and only behind to USA, Brazil and Argentina. At present (2011-12) in India, soybean is being grown in 10.18 million hectare producing 12.28 million tons with seed yield of 1,207 kg per ha. In Madhya Pradesh, it is grown in 5.67 million hectare

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with 6.28 million tonnes of production and 1,108 kg per ha of seed yield (Agricultural Statistics at a Glance, 2012). Owing to its oil and protein profile, this crop has an important role in nutritional security of masses. The yield level in soybean is hovering around 1.2 tons per ha, which is quite low. Yield is a complex entity influenced by several phenological, physiological, yield traits and environment in soybean as true in other crops also. At JNKVV, Jabalpur, several advanced generation fixed genotypes have been developed of different crosses with concerted efforts mounting appropriate selection pressure. Hence, it becomes imperative to know the status of variability, heritability, genetic advance, association and direct and indirect effects of various traits under study in these isolated lines for the assessment of genetic improvement. In view of above aspects, the present investigation was conducted on advanced generation fixed lines of soybean. The experimental material comprised of 26 fixed advanced generation genotypes of soybean.

MATERIAL AND METHODS

The experimental material was comprised of 26 fixed advanced generation genotypes of soybean (JS 97-52 x JSM-299, JS 97-52 x JSM-299, JS 99-76 x JSM-275, JS 97-52 x JS 95-60, JS 97-52 x JS 95-60, JS 97-52 x JSM 120A, JS 97-52 x JSM 286, JS 97-52 x JSM 286, JS 97-52 x JS 20-02, JS 97-52 x JS 95-56, JS 97-52 x JS 95-56, JS 97-52 x JS 95-56, JS 97-52 x JSM-52, JS 97-52 x JSM-52, JSM 146 x JSM-152, JSM 146 x JSM-152, JSM-240 x JSM-189, JS 97-52 x JS(IS) 90-5-12-1, JS 97-52 x JSM-52, JSM-240 x JSM-189, JSM-240 x JSM-189, JS 98-63 x PK-768, JSM 110 x JSM-60, NRC-2007-A-2-3, NRC-2008-B-2-6-2 and NRC-2009-A-1-3-3-1-1). The minimum and maximum temperatures ranged between 22 ^{0}C to 35 ^{0}C, respectively. The experiment was laid out in a randomized complete block design with two replications at the Seed Breeding Farm, Department of Plant Breeding and Genetics, College of Agriculture, JNKVV, Jabalpur during the *kharif* season 2011-12. Size of plot was 3.0 m x 1.6 m with 4 rows at row to row distance of 40 cm. Eleven traits including yield traits and phenological traits were recorded on the basis of five random competitive plants selected from each line in each replication. The mean data of 5 plants were subjected to genotypic correlations which were computed by following the procedure of Miller *et al.* (1958) whereas path coefficient analysis was conducted according to Dewey and Lu (1959).

RESULTS AND DISCUSSION

Estimates of various parameters (heritability, genetic advance, range of variability and coefficients of variation) for assessment of genetic variability (Table 1) were analyzed for the traits which are directly affecting the seed yield. A relative study on comparison of magnitude of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for different traits revealed higher PCV than that of GCV for all the characters under study. It interpreted that number of branches per plant (32.05 % and 29.40 %)

recorded the highest PCV and GCV followed by number of seeds per plant (31.87 % and 28.65 %), seed yield per plant (30.63 % and 27.68 %), number of pods per plant (27.67 % and 25.98 %), biological yield per plant (27.55 % and 24.78 %) and moderate PCV and GCV were recorded for plant height (18.29 % and 17.23 %), 100 seed weight (16.93 % and 16.17 %), number of seeds per pod (13.87 % and 10.45 %), harvest index (12.28 % and 10.84 %) and vegetative phase (10.12 % and 9.91 %).

Table 1. Parameters of genetic variability for phenological and yield components in advanced generations of soybean

Character	Mean	Range		PCV (%)	GCV (%)	h^2b (%)	GA as % of mean 5%
Vegetative phase (days)	40.06	29.00	46.00	10.12	9.91	95.87	19.98
Reproductive phase (days)	56.37	49.50	62.00	5.59	4.64	68.86	7.92
Plant height (cm)	45.30	28.50	59.30	18.29	17.23	88.75	33.43
Number of branches/ plant	3.69	0.50	5.00	32.05	29.40	84.18	55.57
Number of pods/plant	64.77	21.90	83.80	27.67	25.98	88.18	50.26
Number of seeds/plant	127.23	34.50	191.50	31.87	28.65	80.79	53.04
Number of seeds/pods	1.94	1.45	2.52	13.87	10.45	56.74	16.22
Biological yield/ plant (g)	22.87	8.39	29.72	27.55	24.78	80.91	45.91
Harvest Index (%)	50.41	30.77	58.15	12.28	10.84	77.96	19.72
100 seed weight (g)	9.26	7.02	12.47	16.93	16.17	91.15	31.79
Yield/plant (g)	11.70	2.56	15.94	30.63	27.68	81.66	51.53

These results corroborates the findings of Kausar (2005) for plant height and number of branches per plant, , Aditya *et al.* (2012) for number for number of seeds per pod of pods per plant, biological yield per plant and yield per plant, Nehru *et al.* (1999) for number of seeds per plant, Karad *et al.* (2005), Parameshwar (2006) for number of seeds per pod and Banger *et al.* (2003) for 100 seed weight. Devine *et al*. (2002) reported similar findings for plant height and days to maturity.

Heritability is a reliable measure of genetic improvement under selection for polygenic traits and involves total genetic variance which consists of additive, dominance and epistatic variances. The highest heritability was obtained for vegetative phase (95.87 %) followed by 100 seed weight (91.15 %), plant height (88.75 %), number of pods per plant (88.18 %), number of branches per plant (84.18 %), seed yield per plant (81.66 %), biological yield per plant (80.91 %), number of seeds per plant (80.79 %) and harvest index (77.96 %). Moderate heritability was recorded for reproductive phase (68.86 %) and number of seeds per pod (56.74 %).

The highest genetic advance as percentage of mean (at 5 % selection intensity) were recorded for number of branches per plant (55.57 %) followed by number of seeds per plant (53.04 %), seed yield per plant (51.53 %), number of pods per plant (50.26 %), biological yield per plant (45.91 %), moderate for plant height (33.43 %) and 100 seed weight (31.79 %).

Heritability estimates along with genetic advance are normally more helpful in predicting the genetic gain under selection. Number of branches per plant, number of pods per plant, number of seeds per plant, biological yield per plant and seed yield per plant showed high heritability and high genetic advance whereas, high heritability with moderate genetic advance was found for plant height and 100 seed weight indicating the preponderance of additive gene action. Vegetative phase, reproductive phase, number of seeds per pod and harvest index recorded high or moderate heritability with low genetic advance, exhibiting non-additive gene action in expression of their effects. High heritability and genetic advance revealed additive gene action which can be exploited to achieve transgressive segregants adopting appropriate breeding methods. These findings are in agreement with those of Islam and Mian (2008), Nag *et al*. (2007) for plant height, number of pods per plant and number of seeds per plant, Parameshwar (2006) for number of branches per plant and number of seeds per pod, Aditya *et al*. (2011) for biological yield per plant and Gohil *et al*. (2006) for seed yield per plant.

Correlation coefficient

Genotypic correlation coeffi-cients were higher in magnitude than phenotypic correlation coefficients (Table 2). Genotypic correlation coeffi-cient of seed yield per plant was studied with different yield contributing characters. Seed yield per plant exhibited positive and highly significant correlation with biological yield (0.978), number pods per plant (0.845), number of seeds per plant (0.836), plant height (0.753), number of branches per plant (0.742), harvest index (0.735), vegetative phase (0.588) and number of seeds per pod (0.558). Similar, trends were observed in phenotypic correlations as seed yield per plant exhibited positive and highly significant correlation with biological yield (0.962), number pods per plant (0.826), number of seeds per plant (0.825), plant height (0.712), number of branches per plant (0.658), harvest index (0.663), vegetative phase (0.524) and number of seeds per pod (0.487). Present findings revealed that by making selection and improvement for a particular character simultaneous improvement in the associated character(s) may be achieved. This suggested that these characters should be kept in mind provided the characters show high variability while selecting for improvement in seed yield. None of the characters under study exhibited negative correlation with yield. In agreement with

Table 2. Genotypic and phenotypic correlation for phenological and yield components in advanced generations of soybean

Character		Reproductive phase (days)	Plant height (cm)	Branches (No/ plant)	Pods (No/ plant)	Seeds (No/ plant)	Seeds (No/ pod)	Biological yield (g/plant)	Harvest Index (%)	100 seed weight (g)	Yield (g/plant)
Vegetative	**G**	-0.570	0.532*	0.743***	0.823***	0.837***	0.465	0.602**	0.410	-0.558	0.588**
phase (days)	**P**	-0.510***	0.489***	0.684***	0.761***	0.750***	.359**	0.533***	0.363**	-0.519	0.524***
Reproductive	**G**		-0.067	-0.120	-0.035	-0.065	-0.019	0.274	0.017	.530**	0.237
phase (days)	**P**		-0.083	-0.110	-0.058	-0.121	-0.110	0.137	-0.016	.422**	0.107
Plant height	**G**			0.692	0.722***	0.726***	0.432	0.737***	0.506**	0.012	0.753***
(cm)	**P**			0.592***	0.711***	0.697***	0.349*	0.716***	0.395**	-0.026	0.712***
Branches (No/	**G**				0.821	0.718***	0.211	0.734***	0.546**	-0.097	0.742***
plant)	**P**				0.733***	0.656***	0.245	0.623***	0.505***	-0.136	0.658***
Pods	**G**					0.957	0.458*	0.856***	0.574**	-0.278	0.845***
(No/plant)	**P**					0.914***	0.332*	0.842***	0.484***	-0.272	0.826***
Seeds	**G**						.689**	0.852***	0.583*	-0.359	0.836***
(No/plant)	**P**						0.674***	0.823***	0.519***	-0.341*	0.825***
Seeds	**G**							0.542**	0.556**	-0.218	0.558**
(No/pods)	**P**							0.432	0.467***	-0.199	0.487***
Biological	**G**								0.598**	0.116	0.978***
yield (g/plant)	**P**								0.463***	0.091	0.962***
Harvest Index	**G**									0.286	0.735***
(%)	**P**									0.239	0.663***
100 seed	**G**										0.180
weight (g)	**P**										0.148

*Significance levels 0.05** ; 0.01***; 0.005**; 0.001***, if correlation (r) = 0.2732; 0.3541; 0.3836; 0.4432*

Table 3. Genotypic and phenotypic path coefficient analyses showing direct and indirect effects for phenological and yield components in advanced generations of soybean

Character		Veget-ative phase (days)	Repro-ductive phase (days)	Plant height (cm)	Branc-hes (No/ plant)	Pods (No/ plant)	Seeds (No/ plant)	Seeds (No/ pods)	Biological yield (g/ plant)	Harvest Index (%)	100 seed weight (g)
Vegetative phase (days)	**G**	**0.112**	-0.064	0.060	0.084	0.093	0.094	0.052	0.068	0.046	-0.063
	P	**-0.092**	0.047	-0.045	-0.063	-0.070	-0.069	-0.033	-0.049	-0.034	0.048
Reproductive phase (days)	**G**	-0.115	**0.202**	-0.014	-0.024	-0.007	-0.013	-0.004	0.055	0.003	0.107
	P	0.011	**-0.021**	0.002	0.002	0.001	0.003	0.002	-0.003	0.000	-0.009
Plant height (cm)	**G**	0.145	-0.018	**0.273**	0.189	0.197	0.198	0.118	0.201	0.138	0.003
	P	0.003	-0.001	**0.006**	0.003	0.004	0.004	0.002	0.004	0.002	-0.000
Branches (No/plant)	**G**	-0.272	0.044	-0.253	**-0.366**	-0.300	-0.263	-0.077	-0.268	-0.200	0.035
	P	0.025	-0.004	0.022	**0.037**	0.027	0.024	0.009	0.023	0.019	-0.005
Pods (No/plant)	**G**	-2.611	0.110	-2.293	-2.606	**3.174**	-3.037	-1.454	-2.717	-1.822	0.882
	P	-0.542	0.042	-0.507	-0.523	**0.713**	-0.652	-0.237	-0.600	-0.345	0.194
Seeds (No/ plant)	**G**	1.558	-0.122	1.350	1.336	1.780	**1.861**	1.281	1.586	1.086	-0.668
	P	0.583	-0.094	0.541	0.509	0.710	**0.777**	0.523	0.639	0.403	-0.265
Seeds (No/ pods)	**G**	-0.710	0.028	-0.660	-0.323	-0.700	-1.052	**-1.528**	-0.828	-0.850	0.332
	P	-0.134	0.041	-0.130	-0.091	-0.124	-0.251	**-0.373**	-0.161	-0.174	0.074
Biological yield (g/plant)	**G**	1.337	0.608	1.637	1.630	1.900	1.892	1.203	**2.220**	1.328	0.258
	P	0.503	0.129	0.676	0.588	0.795	0.777	0.408	**0.944**	0.437	0.086
Harvest Index (%)	**G**	0.539	0.022	0.665	0.718	0.755	0.767	0.731	0.787	**1.315**	0.376
	P	0.134	-0.006	0.146	0.186	0.178	0.191	0.173	0.171	**0.369**	0.088
100 seed weight (g)	**G**	0.604	-0.574	-0.013	0.105	0.301	0.389	0.236	-0.126	-0.310	**-1.083**
	P	0.033	-0.027	0.002	0.009	0.017	0.022	0.013	-0.006	-0.015	**-0.064**

R Square = 0.9920   Residual effect = 0.0893 the present findings, Burli *et al.* (2010) for yield with plant height and number of pods per plant, Aditya *et al.* (2011) for yield with number of branches per plant, number of pods per plant and harvest index have earlier reported positive correlations.

Path coefficient analysis

Path coefficient analysis was carried out using seed yield per plant as a dependent variable. Phenotypic and genotypic path analyses of different traits (Table 3) revealed that number of pods per plant (0.713 and 3.174), biological yield per plant (0.944 and 2.220), number of seeds per plant (0.777 and 1.861) and harvest index (0.369 and 1.315) depicted substantial phenotypic and genotypic direct effect on seed yield. These traits have also shown positive indirect effect *via* each other which indicated that simultaneous improvement of these traits and ultimately yield can be improved. Similar findings have been reported by Islam and Mian (2008) for plant height and number of seeds per plant, Sirohi *et al.* (2007) for biological yield per plant, number of pods per plant and number of branches per plant.

The study suggests that number of pods and seeds per plant, biological yield, harvest index and 100 seed weight should be given more emphasis while determining the breeding strategies for desirable yield improvement.

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Residual Relative Heterosis and Heterobeltiosis for Different Agro- morphological Traits in Early Segregating Generations of Soybean [*Glycine max* (L.) Merrill] Crosses

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ABSTRACT

The present study was initiated with the objective to estimate the magnitude and direction of residual heterosis for yield and its components in 15 F_3 crosses of soybean. Expression of residual relative heterosis and heterobeltiosis for different yield components were found specific to parental combinations. Residual relative heterosis for trait seed yield per plant was maximum in cross PS 1241 × PS 1042 (78.1 %) followed by Kalitur × PK 472 (68.5 %) and UPSM 534 × PK 472 (60.0 %). Similarly, heterobeltiosis was found maximum in cross PS 1241 × PS 1042 (76.8 %) followed by UPSM 534 × PK 472 (46.6 %) and Kalitur × PK 472 (43.2 %). To get early maturing and high yielding segregants cross PK 416 × VLS 47 was found promising as it exhibited significant residual heterosis over both mid and better parent for earliness and seed yield per plant. Three cross combinations namely, PS 1241 × PS 1042, T 49 × PK 472 PS and 1241 × PK 1162 manifested residual heterosis over mid parent and two crosses namely PS 1241 × PS 1042 and PS 1241 × PK 317 over better parent for seed yield as well as for multiple yield components so these crosses can be advanced to get promising pure lines in future.

Key words: F_3 generation, residual relative heterosis and heterobeltiosis, soybean [*Glycine max* (L.) Merrill], yield components

Soybean [*Glycine max* (L.) Merrill] is one of the most important crops in the world these days. It is considered as 'miracle bean' because of its versatile end uses. It contains 40 per cent high quality protein and 20 per cent cholesterol free oil in addition to other essential amino acids, vitamins and minerals. Soybean oil

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contains about 60 per cent polyunsaturated fatty acid, *viz.* linoleic (50 %) and linolenic acid (8-10 %) in its seed. It is a valuable oilseed crop grown worldwide on account of its wide range of geographical adaptation, unique chemical composition, good nutritional values and functional health benefits. Soybean has witnessed a phenomenal growth in India in past four decades. At present, in India, soybean is cultivated in an area of 10.18 million hectares with a production of 12.28 million tonnes and a productivity of 1 207 kg per ha (DAC, 2012). Demand for soybean is still rising day by day as it has become major oilseed and also serves as raw material for various industries. In India, it is expected that soybean production will increase as the population and living standards increase (James, 2010). On worldwide basis, growing demand for soybean necessitates varietal improvement. Development of high yielding genotypes with best quality combinations is a common goal of every crop breeding programs. To achieve this objective, heterosis breeding has been commercially exploited in many crops. Brim and Young (1971) suggested the possibility of exploiting heterosis in soybean breeding but usefulness of heterosis breeding remained almost unexploited in soybean mainly because of high degrees of self pollination and lack of male sterile lines, which makes hybrid seed production time consuming, labour intensive and uneconomical. Estimation of heterosis in early generations will help in selecting crosses with high potential to advance desirable segregates in subsequent generations (Shinde and Deshmukh, 1989). Residual heterosis in F_2 and F_3 generation in desirable direction also suggest the predominance of dominant gene action (Yadav and Singh, 2011). Efficient selection for heterotic crosses in the F_2 and/or F_3 generation might produce a higher frequency of high-yielding pure lines than breeding methods that ignore the possibility of dominance (Burton and Brownie, 2006). Identification of promising cross combinations in early segregating generations can allow handling of reasonably large segregating populations derived from a few promising crosses.

Therefore, the present investigation was undertaken to estimate residual mid parent (relative heterosis) and better parent (heterobeltiosis) for different agro-morphological traits in early segregating generation of soybean crosses to select superior crosses which will consequently lead to the development of promising pure lines.

MATERIAL AND METHODS

The field experiment was conducted during *kharif* season at the Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Considering the eco-geographic conditions, Pantnagar is located in the foothills of Himalayan range (*Shivalik Hills*) and falls under humid sub-tropical climatic zone in a narrow belt called *'Tarai'*. Geographically it is situated at 29.5^0 North latitude, 79.3^0 East longitude and at an altitude of 243.84 meters above the mean sea level. The soil texture of the experimental site was sandy loam.

In the present investigation, 16 parental lines and 15 F_3 crosses derived from hybridization of these parents in different combinations were sown in randomized block design with two replications for evaluation of different agro-morphological traits. The crosses were grown in ten rows and parents were grown in two rows of three meter length per replication. Row to row and plant to plant distance was maintained at 60 and 5 cm, respectively. All the recommended crop management practices were followed to raise a healthy crop.

The data were recorded on different agro-morphological traits. Observation on days to flowering was recorded at 50 per cent of flowering on whole plot basis. Plant height (cm), number of primary branches per plant, number of pods per plant, basal pod height (cm), number of nodes per plant, dry matter weight per plant (g), and seed yield per plant (g) were recorded on 5 plants sampled randomly from each replication. Number of seed per pod was observed on randomly selected 10 pods from each selected plant and then their mean was calculated and 100 seed weight (g) was recorded from seeds taken from bulk seeds of randomly selected plants for each genotype. Biological yield per plant represents dry matter accumulation of a plant system while seed yield efficiency (%) is ratio of grain yield and non seed dry matter which is expressed in per cent. Harvest index (HI) was determined as economic yield expressed as percentage of total biological yield (Donald, 1962).

Residual heterosis is the amount of heterosis shown by F_2 and subsequent segregating generations. Residual relative heterosis and heterobeltiosis in F_3 generation was estimated as the percentage of deviation of generation mean of F_3 from mid and better parent value, respectively. The significance of residual heterosis was worked out with t-test (Roy, 2000).

RESULTS AND DISCUSSION

In the present study, residual mid and better parent heterosis in desired direction was found in all the evaluated traits. Among them, maximum number of F_3 crosses exhibited residual relative heterosis and heterobeltiosis for trait seed yield per plant (10 and 8) followed by dry matter weight per plant (7 and 4), number of pods per plant (5 and 3) and number of nodes per plant (4 and 3) (Table 1). Residual heterosis for days to 50 per cent flowering ranged from -8.5 to 15.7 and -7.2 to 21.3 per cent over mid and better parent, respectively. Significant negative residual relative heterosis for days to 50 per cent flowering was observed in crosses *viz*., PK 1029 × PK 1162 (-8.5 %) and PK 416 × VLS 47(-7.6 %) over mid parent and cross PK 416 × VLS 47 exhibited significant negative residual heterosis (- 7.2%) over better parent (early parent). Negative residual heterosis for days to 50 per cent flowering is a sign of earliness and early flowering genotypes are generally considered physiologically more efficient than late flowering genotypes and this trait is desirable for moisture stress situation as well as in multiple cropping systems.

Heterosis for reduction of the number of days to flowering was also found by Gadag and Upadhyaya (1995).

The most important attribute of a plant is its yielding ability. In the present investigation, significant positive residual heterosis over mid parent was recorded in ten crosses while positive residual heterosis over better parent was observed in eight crosses for seed yield per plant. Residual relative heterosis was maximum in cross PS 1241 × PS 1042 (78.1 %) followed by Kalitur × PK 472 (68.5 %) and UPSM 534 × PK 472 (60.0 %). Similarly heterobeltiosis was found maximum in cross PS 1241 × PS 1042 (76.8 %) followed by UPSM 534 × PK 472 (46.6 %) and Kalitur × PK 472 (43.2 %). Nelson and Bernard (1984) evaluated 27 hybrids and found five superiors for seed yield, with values between 13 and 19 per cent in relation to the superior parent. Saimaneerat and Srinivas, (1986) compared 11 F_2 hybrids of soybean with their parents and the respective F_3 progenies and found that F_3 generation were not different from F_2 for yield and yield components indicating that additive gene action played an important role in controlling traits in these crosses.

Most of the crosses with significant mid parent heterosis for seed yield per plant also exhibited mid parent residual heterosis for traits dry matter weight per plant followed by number of pods per plant, seed yield efficiency, basal pod height, and harvest index while residual heterosis over better parent were found mainly for dry matter weight per plant, number of pods per plant, harvest index, seed yield efficiency and nodes per plant along with residual better parent heterosis for seed yield per plant (Table 2). Among all the studied traits dry matter weight per plant was found most important and residual mid parent heterosis for both seed yield and dry matter weight was exhibited by crosses *viz.,* Kalitur × PK 472, UPSM 534 × PK 472, Kalitur × PK 317,T 49 × PK 472 and PK 1029 ×G 2115 while crosses Kalitur × PK 472, UPSM 534 × PK 472 and Kalitur × PK 317 exhibited residual heterosis over better parent. It shows the ability of these genotypes for better partitioning of photosynthates. Efficiency of genotypes for dry matter accumulation is a yield indicator in soybean and showed a more consistent correlation to seed yield and pod yield than other yield components (Oko and Uko 1999).

Majority of cross combinations manifesting residual heterosis for seed yield over mid and better parent also exhibited residual heterosis for multiple yield components. Cross combinations PS 1241 × PS 1042 also showed residual relative heterosis for multiple yield components *viz.*, number of pods per plant (31.3 %), basal pod height (-22.6 %), harvest index (69.8 %) and seed yield efficiency (111.9 %). Similarly, cross T 49 × PK 472 exhibited residual heterosis over mid parent for number of pods per plant (33.1 %), seeds per pod (26.7 %), basal pod height (14.1 %) and dry matter weight per plant (28.2 %). Cross PS 1241 × PK 1162 was found superior to mid parent for number of primary branches per plant (31.8 %), number of pods per plant (48.5 %) and seed yield efficiency (50.2 %). Residual better parent heterosis for multiple yield

Table 1. Soybean crosses exhibited significant residual relative hererosis and heterobeltiosis for different agro-morphological traits in desired direction

Character	Cross combinations exhibited residual relative heterosis and heterobeltiosis			
	Mid-parent heterosis		Better parent heterosis	
	No of Crosses	Cross	No of Crosses	Cross
Days to 50 % flowering	2	PK 1029 x PK 1162 (-8.5 %), PK 416 x VLS 47 (-7.6 %)	1	PK 416 x VLS 47 (-7.2 %)
Plant height	2	[(*G. Soja* x PK 262)] x PK 1029 (36.9 %), PS 1024 x G 2121 (33.4 %)	1	[(*G. Soja*) x PK 262] x PK 1029 (24.0 %)
Primary branches (No/plant)	1	PS 1241 x PK 1162 (31.8 %)	1	PK 416 x VLS 47 (27.6 %)
Pods (No/plant	5	PK 1029 x G 2115 (107.5 %), PS 1241 x PK 1162 (48.5 %), Kalitur x PK 472 (40.6 %), T 49 x PK 472 (33.1 %), PS 1241 x PS 1042 (31.3 %)	3	PK 1029 x G 2115 (58.6 %), UPSM 534 x PK 472 (28.3 %), PS 1241 x PK 317 (19.0 %)
Seeds (No/pod)	1	T 49 x PK 472 (26.7 %)	0	-
Basal pod height (cm)	3	PS 1241 x PS 1042 (-22.6 %), UPSM 534 x PK 472 (-8.7 %), T 49 x PK 472 (17.1 %)	1	PS 1241 x PS 1042 (-19.8 %)
Nodes (No/plant)	4	PS 1024 x G 2121 (21.0 %), Kalitur x PK 472 (15.8 %), Kalitur x PK 317 (13.1 %), PK 1029 x PK 1162 (16.0 %)	3	Kalitur x PK 472 (14.3 %), PK 1029 x PK 1162 (11.6 %), Kalitur x PK 317 (11.4 %)
Hundred seed weight (g)	1	PK 1029 x 1024 (31.8 %)	1	PK 1029 x PS 1024 (30.4 %)
Dry matter (g/plant)	7	Kalitur x PK 472 (61.0 %), PS 1024 x G 2121 (50.7 %), UPSM 534 x PK 472 (33.6 %), Kalitur x PK 317 (29.8 %), T 49 x PK 472 (28.2 %), PK 1241 x Jupiter (21.8 %), PK 1029 x G 2115 (19.1 %)	4	Kalitur x PK 472 (56.2 %), PS 1024 x G 2121 (37.5 %), UPSM 534 x PK 472 (29.4 %), Kalitur x PK 317 (19.0 %)
Harvest index (%)	2	PS 1241 x PK 317 (76.6 %), PS 1241 x PS 1042 (69.8 %)	2	PS 1241 x PK 317 (64.6 %), PS 1241 x PK 1042 (53.9 %)

Seed yield efficiency (%)	3	PS 1241 x PK 317 (121.9 %), PS 1241 x PS 1042 (111.9 %), PS 1241 x PK 1162 (50.2 %)	2	PS 1241 x PK 317 (103.8 %), PS 1241 x PS 1042 (80.5 %)
Seed yield (g/plant)	10	PS 1241 x PS 1042 (78.1 %), Kalitur x PK 472 (68.5 %), UPSM 534 x PK 472 (60.0 %), PS 1241 x PK 317 (56.6 %), PS 1241 x PK 1162 (47.1 %), Kalitur x PK 317 (38.4 %), T 49 x PK 472 (45.3 %), PK 1029 x G 2115 (33.7 %), PK 1241 x Jupiter (25.4 %), PK 416 x VLS 47 (20.3 %)	8	PS 1241 x PS 1042 (76.8 %), UPSM 534 x PK 472 (46.6 %), Kalitur x PK 472 (43.2 %), PS 1241 x PK 317 (37.3 %) PS 1241 x PK 1162 (36.5 %), PK 416 x VLS 47 (29.6 %), T 49 x PK 472 (24.9 %), Kalitur x PK 317 (34.0 %)

components was found in cross PS 1241 × PK 317 for number of pods per plant (19.0 %), harvest index (64.6 %) and seed yield efficiency (103.8 %). Similarly, cross PS 1241 × PS 1042 exhibited maximum highly significant residual better parent heterosis for basal pod height (-19.8 %), harvest index (53.9 %) and seed yield efficiency (80.5 %). To get early maturing and high yielding segregants, cross PK 416 × VLS 47 was found promising as it exhibited significant residual heterosis over both mid and better parent for earliness and seed yield per plant. The estimates of residual heterosis will help in selection of potential cross combinations in early generation as the crosses with high heterotic effects are more likely to produce better performing segregants than those with low heterotic effects (Sagar and Chandra, 1977).

Therefore, chances of obtaining promising segregants from these crosses are higher and selection of potential cross combination in early generation can ultimately save the time and labour involved for breeding.

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Table 2. Soybean crosses exhibited significant residual relative heterosis and heterobeltiosis for seed yield and other associated traits

Cross combination exhibited residual mid and better parent heterosis for seed yield/plant	Residual heterosis with other associated yield components	
PS 1241 x PS 1042	Number of pods/plant, Basal pod height, Harvest index, Seed yield efficiency	Basal pod height, Harvest index, Seed yield efficiency
Kalitur x PK 472	Number of nodes/plant, Dry matter weigh/plant	Number of nodes/plant, Dry matter weigh/plant
UPSM 534 x PK 472	BPH, DMW	Number of pods/plant, Dry matter weigh/plant
PS 1241 x PK 317	Harvest index, Seed yield efficiency	Number of pods/plant, Harvest index, Seed yield efficiency
PS 1241 x PK 1162	Number of primary branches/plant, Number of pods/plant, Seed yield efficiency	-
Kalitur x PK 317	Number of nodes/plant, Dry matter weigh/plant	Number of nodes/plant, Dry matter weigh/plant
T 49 x PK 472	Number of pods/plant, Seeds/pod, Basal pod height, Dry matter weigh/plant	-
PK 416 x VLS 47	Days to 50 % flowering	Days to 50 % flowering, Number of primary branches/plant
PK 1029 x G 2115*	Number of pods/plant, Dry matter weigh/plant	*
PK 1241 x Jupiter*	Dry matter weigh/plant	*

**Cross combinations exhibited residual heterosis for seed yield per plant only over mid parent*

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Productivity of Four Soybean Varieties as Affected by Intercropping with Corn Planting Geometry

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ABSTRACT

Two field trials were carried out at Gemmiza Agricultural Experiments and Research Station, A.R.C., El-Gharbia governorate, Egypt, during 2011 and 2012 seasons to evaluate the suitable soybean variety for intercropping with corn by using high plant densities of both crops for agro-economic feasibility. Corn plants (S.C. 10) were grown in one, two and three plants per hill at 30, 60 and 90 cm hill spacing, respectively, while soybean varieties (Giza 21, Giza 22, Giza 35 and Giza 111) were drilled in two rows (15 cm spaced) on both sides of corn ridge, in addition to solid plantings of both the crops. A split split plot distribution in randomized complete block design with three replications was used. Results indicated that intercropping soybean with corn decreased seed yields per plant by 33.5% and per ha by 48.7% as compared to solid planting of soybean. Soybean variety Giza 22 produced the highest seed yield. Increasing number of corn plants from one to three plants per hill increased seed yields per plant and per ha by 19.5 and 16.8 per cent, respectively. Interactions affected significantly most soybean parameters. LER ranged from 1.32 to 1.64. LEC was above 0.25. Intercropping soybean variety Giza 22 with corn which distributed in three plants per hill increased economic return for Egyptian farmers by about £uro 343 per ha as compared with solid planting of corn. Soybean variety Giza 22 was most compatible with corn plants under mixed intercropping pattern, in addition to 7.65 ton per ha of corn grains was gained by intercropping.

Key words: Intercropping, corn plant geometry, soybean varieties, light interception, LER, LEC, financial returns

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Egypt is facing acute shortage of edible oil. Vegetable oil is imported in large quantities to satisfy the domestic needs of the country. The gap between production and consumption reaches about 85 per cent (FAO, 2012). In the Nile Valley and Delta, oil seed crops cover 1.7 per cent of the cultivated land (Metwally *et al*., 2012). It is not feasible to expand the area for oil seed crops because of high competition from the other summer crops.

However, it is feasible to augment the acreage of these crops such as soybean [*Glycine max* (L.) Merrill] in these lands by inter-cropping with cash crops like corn (*Zea mays* L.). It is recommended to increase total agriculture products in Egypt (Metwally, 1999). Demand for the corn grains in the Egyptian market is intensively increasing where corn cultivated area reached about 679,898 ha in 2011 with an average yield of 1.35 ton per ha, but there is a decline in soybean acreage due to increased production costs and lower net returns as compared with the other strategic summer crops such as corn, rice and cotton. In 1970, the cropped area of soybean was about 1,320 ha with an average seed yield of 0.76 ton per ha. The acreage has increased rapidly, reaching 59,577 ha in 1983 with an average yield of 2.71 ton per ha, then it began to decline continuously till it reached 8,785 ha in 2011 with an average yield of 3.23 ton per ha (Egyptian Bulletin of Statistical Cost Production and Net Return, 2012).

Consequently, there is the need to expand the scope of soybean cultivation through intercropping system. Intercropping has since been recommended as a practical alternative solution in Egypt for increasing national production of soybean (Sayed Galal and Metwally, 1986). Also, intercropping cereals with legumes is usually done to maximize productivity in many part of the Mediterranean region (Aynehband *et al*., 2010).

In Egypt, intercropping is the best way to keep the area of soybeans without significant change in crop structure. Crop species in intercropping pattern must be carefully chosen to minimize competition and enhance the efficient use of water, light and nutrients (Sayed Galal *et al*., 1983). Under intercropping plantings, yield components of corn can be governed by plant density and distribution of these plants in the unit area with regard to soybean variety and its plant density as the competition for environmental resources between them must be less than exists within the same species. Decreasing distance between corn hills resulted in adverse effects on growth and yield of soybean plants (Tetiokagho, 1988 and Metwally *et al*., 2012).

Row arrangement, in contrast to arrangement of component crops within rows, may also influence the productivity of an intercropping system (Mohta and De, 1980). Spatial arrangement of single rows of corn alternating with double rows of soybean recorded the best yields with respect to soybean (Addo- Quaye *et al*., 2011). However, the appropriate distribution for corn plants per unit area under intercropping conditions may minimize the adverse effects. Increasing corn row width from 70 to 140 cm and growing corn in the middle of the wide ridge, while soybean was planted in two drills at each side of corn ridge (four drills/ridge) resulted in the highest land equivalent ratio (LER) as compared with the other patterns (Metwally *et al*., 2012).

Canopy architecture of corn plant can play an important role in intercepting solar energy under the appropriate distribution for corn plants per unit area; it is a commonly used strategy to capture sunlight that would not otherwise be available to the other crop. Under intercropping conditions with corn, light penetration was decreased during soybean canopy development (Foroutan-pour *et al*., 1999). Consequently, corn canopy architecture (spatial distri-bution of shoot organs) plays an important role in the amount of sunlight radiation that is intercepted by other crop under intercropping pattern, and light proved as a critical competition factor in intercropping culture. The reduction in light reaching the legume canopy when intercropped with corn was about 30 – 50 per cent of the total incoming radiation and began around 30 - 35 days after corn seeding (Polthanee and Changsri, 1999 and Polthanee and Treloges, 2002 and 2003). Light intensity within soybean plants at 85 days from soybean sowing was affected significantly by shading of adjacent corn plants. Light intensity at middle and bottom of soybean plants were considerably decreased by 33.9 and 48.8 per cent, respectively, as compared with recommended solid planting of soybean (Metwally *et al*., 2012).

Success of intercropping pattern depends on the ability of the second crop to become established under the canopy of the first crop in variable midsummer conditions. Selection of soybean varieties suitable for intercropping has been restricted to introductions from the U.S. (Sayed Galal *et al*., 1984). These introductions are varieties and lines selected for good performance in solid culture pattern. In general, soybean variety could play an important factor to escape from shading effects of corn plants of different species where there were significant differences between soybean varieties in some parameters of growth, yield and its attributes under intercropping conditions (Metwally *et al*., 2012). The objective of this study was to evaluate the suitable soybean variety for intercropping with corn by using high plant densities of both crops for agro-economic feasibility.

MATERIAL AND METHODS

Two field trials were carried out at Gemmiza Agricultural Experiments and Research Station, A.R.C., El-Gharbia governorate (Lat. 30° 47' 27" N, Long. 30° 59' 53" E, 22 m a.s.l.), Egypt during 2011 and 2012 seasons to evaluate the suitable soybean variety for intercropping with corn by using high plant densities of both crops for agro-economic feasibility.

Corn plants (S.C. 10) were grown in one, two and three plants per hill at 30, 60 and 90 cm hill spacing (one row in middle of ridge), respectively, while four soybean varieties (Giza 21, Giza 22, Giza 35 and Giza 111) were drilled in two rows (15 cm spaced) on both sides of corn ridge (mixed intercropping pattern), in addition to solid plantings of both the crops (Fig. 1). Soybean was thinned to two plants at 15 cm between hills. Soybean seeds were sown on May 22nd and 27th in 2011 and 2012 seasons, respectively, while, corn was sown ten days later.

Corn variety kindly provided by Corn Research Department, Field Crops Research Institute, Agricultural Research Center (ARC). Also, soybean varieties kindly provided by Food Legumes Research Department, Field Crops Research Institute, ARC. In all 12 treatments combinations along with sole corn and soybean were laid out in split split plot design with three replications.

Cropping systems (mixed intercropping and solid) were randomly assigned to the main plots, soybean varieties were allotted in sub-plots and the distributions of corn plants were devoted to sub- sub-plots. The area of sub sub-plot was 14.0 m^2, each plot consisted of 5 ridges, and each ridge was 4.0 m in length and 70 cm in width.

Solid cultures of both crops were used to estimate the competitive relationships. Pure stand of corn ridges was conducted by leaving one plant per hill with distance of 30 cm apart, whereas in case of pure stand of soybean ridges was conducted by drilling two rows per ridge and was thinned to two plants distanced at 15 cm between hills on ridges 70 cm width. Normal recommended cultural practices for growing corn and soybean crops were used.

In all the treatments, the population of corn and soybean was uniformly maintained at 47,619 and 380,952 per ha, respectively. Egyptian clover (berseem) was the preceding winter crop in both seasons. The experimental soil texture was clay. Chemical analysis of the soil, pH value, N, phosphorus and potassium, were analyzed by Water and Soil Research Institute, A.R.C. (Table 1). Chemical analysis of the soil was determined using the methods described by Jackson (1958) and Chapman and Pratt (1961).

Table 1. Chemical properties of Gemmiza site in 2011 and 2012 seasons before sowing soybean plants

Chemical properties	Growing season	
	2011	2012
pH	7.90	7.95
Available N ppm (Optimum level: 40 – 80)	80.0	100.0
Available P ppm (Optimum level: 10 – 15)	2.00	3.00
Available K ppm (Optimum level: 300 – 500)	150.0	175.0

The parameters on vegetative growth at 85 days of soybean sowing recorded on five plants from each plot were light intensity (lux) inside each canopy (at the middle of the plant and at the bottom of the plant at 20 cm from the soil surface) by Lux-meter apparatus at 12 h and expressed as percentage from light intensity measured above the plant, dry weight per plant, dry weight of leaves per plant and dry weight of pods per plant. Data on yield (ton/ha) and yield attributes (plant height, seed index and seed yield per plant).

Crude oil and protein contents in the seed were done with the support of Forage Research Department, ARC according to procedures described by

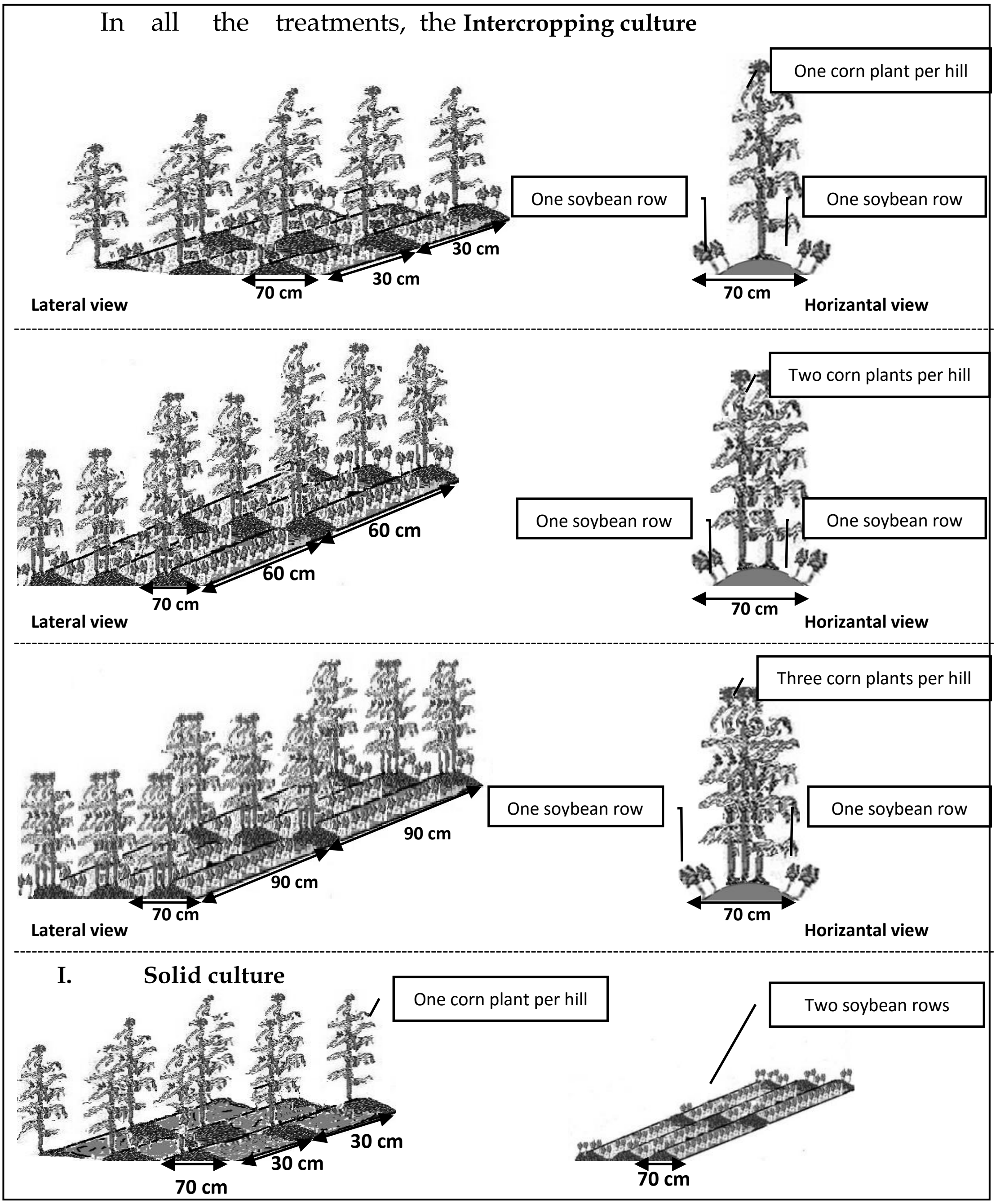


Figure 1. Intercropping corn and soybean as compared with solid plantings

A.O.A.C. (1995). Crude protein content was calculated by multiplying total nitrogen by 5.71 (Sadasivam and Manickam, 1996).

Competitive relationship, Land Equivalent Ratio (LER) (Mead and Willey, 1980) and Land Equivalent Coefficient (LEC) (Adetiloye *et al.*, 1983) were calculated as follows.

LER = (Y_{ab} / Y_{aa}) + (Y_{ba} / Y_{bb})
Where Y_{aa} = Pure stand yield of crop a (corn), Y_{bb} = Pure stand yield of crop b (soybean), Y_{ab} = Intercrop yield of crop a (corn), Y_{ba} = Intercrop yield of crop b (soybean)
LEC = L_a x L_b
Where La = LER of crop a (corn), L_b = LER of crop b (soybean)

Farmer's benefit (£uro) was calculated as a difference between total net returns from intercropping cultures and solid ones (Metwally *et al.*, 2009).

Corn grains and soybean seeds prices presented by Egyptian Bulletin of Statistical Cost Production and Net Return (2012) were used. Net returns were calculated by subtraction the sum of fixed cost of corn plus variable costs of both the crops according to intercropping pattern.

Analysis of variance of the obtained results of each season was performed. The homogeneity test was conducted of error mean squares and accordingly, the combined analysis of the two experimental seasons was carried out. The measured variables were analyzed by ANOVA using MSTATC statistical package (Freed, 1991). Mean comparisons were done using least significant differences (L.S.D) method at 5 per cent level of probability to compare differences between the means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Vegetative growth of soybean plants

Cropping systems: Intercepted light intensity within soybean plants, leaves and pods dry weights per plant were affected significantly by cropping systems, whereas, branches dry weight per plant was not affected at 85 days from soybean sowing (Tables 2 and 3). Solid planting of soybean had higher values for light intensity within soybean plants, leaves and pods dry weights per plant than intercropped soybean. Shading of adjacent corn plants led to decrease in light intensity at middle and bottom of soybean plants by 39.9 and 45.7 per cent, respectively, as compared to solid planting of soybean (Table 2). Also, dry weights of leaves and pods per plant of intercropped soybean were reduced by 28.2 and 17.7 per cent, respectively as compared to solid culture of soybean. Dry weight of branches per plant in intercrops was statistically equal to those of soybean solid culture (Table 3).

It is clear that intercropping corn with soybean resulted in decreasing light interception by soybean plants and consequently negative effect on photosynthesis process during different periods of soybean growth. Mixed intercropping pattern resulted in unfavorable conditions for growth of soybean plant which reflected on the little dry matter accumulation in different parts of soybean organs as compared with solid culture of soybean. These results are in agreement with those reported by Bowes *et al.* (1972) who investigated that higher light intensities during growth of 'Wayne' soybeans resulted in increases in photosynthesis rate, light saturation intensity, ribulose-1,5-bisphosphate carboxylase activity and specific leaf weight.

Plant dry matter production often shows a positive correlation with the amount of intercepted radiation by crops in intercropping system (Sivakumar and Virmani, 1980) and sole cropping (Kiniry *et al.*, 1989). Also, Board and Harville (1992) mentioned that spatial arrangement of intercropping culture resulted in shading effects of adjacent corn plants on soybean plant, where, environmental conditions prevailing during growth period, especially intensity and quality of intercepted solar radiation by canopy, are important determinants of yield components and hence yield of soybean.

On the other hand, low light levels available for shaded soybean plants might have caused a restriction of their genetic potential resulting in the modification of their growth pattern (Odeleye *et al.*, 2001). Spatial distribution in the field is of great importance when intercropping two or more species, since it affects the efficiency with which solar radiation and space are utilized. Spatial arrangement has an important influence on the degree of competition between crops (Addo-Quaye *et al.,* 2011). Finally, Metwally *et al.* (2012) investigated that growing corn with soybean decreased light intensity at middle and bottom of soybean plants by

Table 2. Effect of cropping systems, soybean varieties, distributions of corn plants and their interactions on intercepted light intensity within soybean plants at 85 days from soybean sowing (combined data across 2011 and 2012 seasons)

Parameters/ Cropping systems	Distributions of corn plants	Percentages of light intensity (Lux) at middle of the plant: Soybean varieties Giza 21	Giza 22	Giza 35	Giza 111	Mean	bottom of the plant: Soybean varieties Giza 21	Giza 22	Giza 35	Giza 111	Mean
Intercropping culture	One plant/hill	4.52	5.22	4.95	4.14	**4.70**	1.52	1.93	1.66	1.37	**1.62**
	Two plants/hill	5.29	5.94	5.61	5.01	**5.46**	2.13	2.54	2.28	1.98	**2.23**
	Three plants/hill	5.98	6.69	6.34	5.81	**6.20**	2.78	3.15	2.98	2.60	**2.87**
Average of intercropping		**5.26**	**5.95**	**5.63**	**4.98**	**5.45**	**2.14**	**2.54**	**2.30**	**1.98**	**2.24**
Solid culture of soybean varieties		8.98	9.31	9.18	8.82	**9.07**	4.02	4.54	4.21	3.78	**4.13**
General mean of soybean varieties		**7.12**	**7.63**	**7.40**	**6.90**	**7.26**	**3.08**	**3.54**	**3.25**	**2.88**	**3.18**
L.S.D. at 5% of cropping systems (S)						1.31					1.17
L.S.D. at 5% of soybean varieties (V)						0.59					0.47
L.S.D. at 5% of distributions of corn plants (D)						0.42					0.33
L.S.D. at 5% of S x V						1.48					1.36
L.S.D. at 5% of S x D						1.83					1.69
L.S.D. at 5% of V x D						0.74					0.62
L.S.D. at 5% of S x V x D						2.32					2.16

33.9 and 48.8 per cent, respectively, which led to decrease in dry weight of soybean plant.

Soybean varieties: Soybean varieties differed significantly in intercepted light intensity within soybean plants, leaves and pods dry weights per plant, whereas, branches dry weight per plant was not differed at 85 days from soybean sowing (Tables 2 and 3).Soybean variety Giza 22 had the highest values of light intensity within soybean plants and pods dry weight per plant, whereas, Giza 111 had the highest value of leaves dry weight per plant as compared with the other varieties. This may be due to canopy structure of soybean variety Giza 22 having narrow leaves which reflected on the low shading around soybean plant and consequently more solar radiation to the other parts of soybean plant, whereas, the other soybean varieties allowed passage little solar radiation to the other parts of soybean plant because of soybean varieties, *i.e.* Giza 21 is the tallest (Noureldin *et al.*, 2002) and Giza 111 have broad leaves (Metwally *et al.*, 2012) as compared to the other soybean varieties.

These results imply that soybean varieties responded differently for the studied vegetative growth parameters. These results are in a good line with those obtained by Lindermann and Ham (1979) who reported that there were significant varietal differences in soybean in relation to dry matter. Also, Seversike *et al.* (2009) indicated that seven-leaflet isolines of soybean had 10 to 21 per cent greater cumulative intercepted photo-synthetically active radiation (PAR) at populations 40 m^2 as compared to three-leaflet isolines. Moreover, Metwally *et al.* (2012) found that soybean variety Giza 22 had higher values for intercepted light intensity with soybean plants than the other variety (Giza 111). Finally, Solomon *et al.* (2012) found that soybean variety EthioYugoslavia produced significantly higher amount of dry matter (23.75 g/plant) than Jalele and Cheri which recorded 19.03 and 17.22 g per plant dry matter, respectively. However, the varieties Jalele and Cheri were statistically at par in terms of dry matter production.

Distributions of corn plants: Tripling number of corn plants from one to three plants per hill affected significantly intercepted light intensity within soybean plants, leaves and pods dry weights per plant, whereas, branches dry weight per plant was not affected at 85 days from soybean sowing (Tables 2 and 3). Increasing distance between corn hills from 30 to 90 cm increased light intensity at middle and bottom of soybean plants by 31.9 and 77.1 per cent, respectively. Moreover, dry weights of leaves and pods per plant were increased by increasing number of corn plants from one to three plants per hill. Increasing number of corn plants from one to three plants per hill increased dry weights of leaves and pods per plant by 17.7 and 9.5 per cent, respectively.

These results indicated that corn plant geometry which distributed at 90 cm hill spacing formed suitable spatial arrangement of mixed intercropping pattern which decreased inter-specific competition between corn and soybean

Table 3. Effect of cropping systems, soybean varieties, distributions of corn plants and their interactions on dry weights of branches, leaves and pods per plant at 85 days from soybean sowing (combined data across 2011 and 2012 seasons)

Parameters/ Cropping systems	Distributions of corn plants	Dry weight (g) per plant														
		Branches					Leaves					Pods				
		Soybean varieties				Mean	Soybean varieties				Mean	Soybean varieties				Mean
		Giza 21	Giza 22	Giza 35	Giza 111		Giza 21	Giza 22	Giza 35	Giza 111		Giza 21	Giza 22	Giza 35	Giza 111	
Inter-cropping culture	One plant/hill	6.82	6.51	6.42	6.90	**6.66**	5.22	5.10	3.39	5.88	**4.89**	9.34	13.23	12.81	12.36	**11.93**
	Two plants/hill	6.98	6.79	6.59	7.09	**6.86**	5.71	5.59	3.92	6.14	**5.34**	9.98	13.68	13.47	12.95	**12.52**
	Three plants/hill	7.28	7.03	6.93	7.32	**7.14**	6.26	5.91	4.31	6.56	**5.76**	10.63	14.11	13.78	13.77	**13.07**
Average of intercropping		**7.02**	**6.77**	**6.64**	**7.10**	**6.88**	**5.73**	**5.53**	**3.87**	**6.19**	**5.33**	**9.98**	**13.67**	**13.35**	**13.02**	**12.50**
Solid culture of soybean varieties		7.49	7.24	7.18	7.67	**7.39**	7.89	7.33	6.24	8.27	**7.43**	12.15	16.44	16.12	16.09	**15.20**
General mean of soybean varieties		**7.25**	**7.00**	**6.91**	**7.38**	**7.13**	**6.81**	**6.43**	**5.05**	**7.23**	**6.38**	**11.06**	**15.05**	**14.73**	**14.55**	**13.85**
L.S.D. at 5% of cropping systems (S)						NS					1.73					2.16
L.S.D. at 5% of soybean varieties (V)						NS					1.06					1.64
L.S.D. at 5% of distributions of corn plants (D)						NS					0.72					1.10
L.S.D. at 5% of S x V						NS					1.96					2.31
L.S.D. at 5% of S x D						NS					2.18					2.58
L.S.D. at 5% of V x D						NS					1.21					1.95
L.S.D. at 5% of S x V x D						NS					2.34					2.86

plants as compared with the other distances. Three corn plants which were grown at 90 cm hill spacing allowed passage more solar radiation to the other adjacent soybean plants which affected positively photosynthesis process and consequently more dry matter accumulation in different parts of soybean plant organs (Table 3).

These data show that soybean intercropped with corn under the wide distance (90 cm) had favorable conditions for growth of the different parts of shaded soybean plant (leaves and pods dry weights) as compared with the other distances. These results are in agreement with those reported by Metwally *et al.* (2012) who indicated that wide spaced corn hills formed a good chance for intercropped soybean plants to intercept high percentage of solar radiation which led to significant increase in plant dry weight.

Interactions among cropping systems, soybean varieties and distributions of corn plants: With respect to response of soybean varieties to cropping systems, intercepted light intensity within soybean plants, leaves and pods dry weights per plant reached the 5 per cent level of significance, whereas, branches dry weight per plant did not reach the 5 per cent level of significance at 85 days from soybean sowing (Tables 2 and 3). Solid planting of soybean variety Giza 22 recorded the highest values of intercepted light intensity within soybean plants and pods dry weight per plant , while, the lowest values of intercepted light intensity were obtained by intercropping corn with soybean variety Giza 111. Intercropping corn with soybean variety Giza 35 produced the lowest leaves dry weight per plant, while, the lowest pods dry weight per plant was obtained by intercropping corn with soybean variety Giza 21. This implies that improved varieties of soybean responded differently to cropping system.

With respect to response of distributions of corn plants to cropping systems, intercepted light intensity within soybean plants, leaves and pods dry weights per plant reached the 5 per cent level of significance, whereas, branches dry weight per plant did not reach the 5 per cent level of significance at 85 days from soybean sowing (Tables 2 and 3). Solid planting of soybean recorded the highest values of intercepted light intensity within soybean plants and pods dry weight per plant, while, the lowest values of intercepted light intensity, leaves and pods dry weights per plant were obtained by intercropping soybean with corn under narrow distance between hills (30 cm).

With respect to response of soybean varieties to distributions of corn plants, intercepted light intensity within soybean plants, leaves and pods dry weights per plant reached the 5 per cent level of significance, whereas, branches dry weight per plant did not reach the 5 per cent level of significance at 85 days from soybean sowing (Tables 2 and 3). Intercropping soybean variety Giza 22 with corn which grown at wide distance between hills (90 cm) recorded the highest values of intercepted light intensity within soybean plants and pods dry weight per plant, while, the lowest values of intercepted light intensity were obtained by intercropping soybean variety Giza 111 with corn which grown at narrow distance between hills (30 cm). Intercropping soybean variety Giza 35 with one corn plant per hill produced the lowest leaves dry weight per plant, while, the lowest pods dry weight per plant was obtained by intercropping soybean variety Giza 21 with corn under 30 cm hill spacing. These data reveal that soybean varieties x distributions of corn plants interaction was observed for pods dry weights per plant suggesting that some soybean varieties were performed better at the highest corn plant distance (90 cm). With respect to response of cropping systems and soybean varieties to distributions of corn plants, intercepted light intensity within soybean plants, leaves and pods dry weights per plant reached the 5 per cent level of significance, whereas, branches dry weight per plant did not reach the 5 per cent level of significance at 85 days from soybean sowing (Tables 2 and 3). Solid planting of soybean variety Giza 22 recorded the highest values of intercepted light intensity within soybean plants and pods dry weight per plant, while, the lowest values of intercepted light intensity were obtained by intercropping soybean variety Giza 111 with corn which grown at narrow distance between hills (30 cm). Intercropping soybean variety Giza 35 with one corn plant per hill produced the lowest leaves dry weight per plant, while, the lowest pods dry weight per plant was obtained by intercropping soybean variety Giza 21 with corn under 30 cm hill spacing.

Soybean yield and its attributes

Cropping systems: Plant height, seed yields per plant and per ha, seed oil and protein contents were affected significantly by cropping systems, whereas, seed index was not affected (Tables 4 and 5). Solid planting of soybean had higher values for seed yields per plant and per ha and seed oil content than intercropping culture. The reverse was true for plant height and seed protein content.

Intercropping corn with soybeans led to an increase in height of soybean plant by 10.5 per cent as compared with intercropped soybean. This may be due to shading effects of adjacent corn plants. It is obvious that mixed intercropping pattern formed unfavorable conditions for soybean plants which reflected on the severe decrease in intercepted light intensity during the early periods of soybean growth and consequently more amounts of plant hormones. So, the observed response in plant height of soybean may be primarily attributed to an increase of internode elongation of shaded soybean plant as a result of increasing plant hormones. So, the observed response in plant height of soybean may be primarily attributed to an increase of internode elongation of soybean plant as a result of increasing plant hormones.

These results are confirmed by Marchiol *et al.* (1992) who indicated that intercropping soybean with corn increased soybean plant height. Shading (49–20 % of ambient light) resulted in lengthening of internodes and increased lodging in soybean plants (Ephrath *et al.*, 1993). Also, Addo-Quaye *et al.* (2011) showed that as the soybean plant becomes taller, self-shading is enhanced and there may be an exceedingly steep light gradient between the top and bottom of the plant. Similar results were reported by Undie *et al.* (2012) who revealed that soybean plant height was increased above its sole crop height at all intercrop arrangements.

Intercropping soybean with corn decreased seed yields per plant and per ha by 33.5 and 48.7 per cent, respectively, as compared to solid planting of soybean (Tables 4 and 5). It is clear that mixed intercropping pattern affected negatively yield attributes of soybean which produced about two-thirds of its seeds of soybean solid culture.

This may be due to adverse effects of intercropping culture which increased inter-specific competition between corn and soybean plants for basic growth resources (Mohta and De, 1980 and Olufajo, 1992) as compared with soybean solid culture. These results are in the same context of those obtained by Egbe (2010) who investigated that intercropped soybean produced lower seed yield than their sole crop counterparts. Also, Metwally *et al.* (2012) showed that intercropping corn with soybean affected negatively on seed yield per plant and consequently seed yield per ha. Also, the observed protein and oil content is explained by the photosynthetic responses of partial shaded and fully exposed leaves. There was a significant decrease and increase of soybean photosynthetic rates of both non-shaded and partially shaded leaves respectively within the intercrops (Akunda, 2001). These results are in agreement with those reported by Proulx and Naeve (2009) who indicated that shade and defoliation treatments each resulted in decreased soybean yield and altered protein and oil concentrations relative to the control.

Soybean varieties: Soybean varieties differed significantly in plant height, seed yields per plant and per ha, seed oil and protein contents, whereas, seed index was not affected (Tables 4 and 5). Soybean variety Giza 22 had higher values of seed yield per plant and per ha and seed oil content, whereas, Giza 111 had the highest seed protein content as compared with the others. This may be due to soybean variety Giza 22 had higher ability to overcome the severe conditions of intercropping culture than the other varieties (Table 2).

Shafik (2000) demonstrated that soybean genotypes differed significantly in yielding ability under solid and intercropping cultures. Similar variability indicating considerable diversity for seed yield per plant was observed by Bharadwaj *et al.* (2007) which evaluated 87 accessions of soybean. Also, Mudita *et al.* (2008) showed that soybean cultivar affected significantly soybean yield per ha in one year. Moreover, Egbe (2010) found that soybean varieties, *i.e.* Samsoy 2 and TGX 923-2E gave significantly higher seed yield than TGX 536-O2D soybean variety. These results are in a good line with those obtained by Metwally *et al.* (2012) who revealed that soybean variety Giza 22 gave the highest seed yield.

Table 4. Effect of cropping systems, soybean varieties, distributions of corn plants and their interactions on plant height, seed index and seed yield per plant at harvest (combined data across 2011 and 2012 seasons)

Parameters/ Cropping systems	Distri-butions of corn plants	Plant height (cm) Soybean varieties Giza 21	Giza 22	Giza 35	Giza 111	Mean	Seed index (g/100 seeds) Soybean varieties Giza 21	Giza 22	Giza 35	Giza 111	Mean	Seed yield per plant (g) Soybean varieties Giza 21	Giza 22	Giza 35	Giza 111	Mean
Inter-cropping culture	One plant/hill	107.0	102.3	100.5	106.9	**104.1**	16.15	16.38	16.37	16.16	**16.26**	5.02	5.91	5.66	4.76	**5.33**
	Two plants/hill	105.3	100.0	96.6	102.8	**101.1**	16.14	16.18	16.26	16.17	**16.18**	5.42	6.54	6.03	5.23	**5.80**
	Three plants/hill	101.9	96.5	93.3	100.2	**97.9**	16.30	16.24	16.28	16.20	**16.25**	5.93	7.26	6.62	5.69	**6.37**
Average of intercropping		**104.7**	**99.6**	**96.8**	**103.3**	**101.0**	**16.19**	**16.26**	**16.30**	**16.17**	**16.23**	**5.45**	**6.57**	**6.10**	**5.22**	**5.83**
Solid culture of soybean varieties		97.4	89.0	88.2	91.3	**91.4**	19.18	19.45	19.27	19.06	**19.24**	8.71	9.02	8.77	8.59	**8.77**
General mean of soybean varieties		**101.0**	**94.3**	**92.5**	**97.3**	**96.2**	**17.68**	**17.85**	**17.78**	**17.61**	**17.73**	**7.08**	**7.79**	**7.43**	**6.90**	**7.30**
L.S.D. at 5% of cropping systems (S)						5.33					N.S.					2.05
L.S.D. at 5% of soybean varieties (V)						2.82					N.S.					0.81
L.S.D. at 5% of distributions of corn plants (D)						2.65					N.S.					0.69
L.S.D. at 5% of S x V						5.58					N.S.					2.18
L.S.D. at 5% of S x D						5.89					N.S.					2.31
L.S.D. at 5% of V x D						3.61					N.S.					1.06
L.S.D. at 5% of S x V x D						6.58					N.S.					2.23

Table 5. Effect of cropping systems, soybean varieties, distributions of corn plants and their interactions on the yield per ha, oil and protein contents of soybean seeds at harvest (combined data across 2011 and 2012 seasons)

Parameters/ Cropping systems	Distributions of corn plants	Soybean seed yield per ha (ton)					Corn grain yield per ha (ton)					Seed content (%) Oil					Protein				
		Soybean varieties				Mean	Soybean varieties				Mean	Soybean varieties				Mean	Soybean varieties				Mean
		Giza 21	Giza 22	Giza 35	Giza 111		Giza 21	Giza 22	Giza 35	Giza 111		Giza 21	Giza 22	Giza 35	Giza 111		Giza 21	Giza 22	Giza 35	Giza 111	
Inter-cropping culture	One plant/hill	1.45	1.66	1.53	1.38	**1.50**	6.37	6.52	6.49	6.24	**6.40**	20.0	20.5	20.3	20.1	**20.2**	45.1	44.2	44.5	45.0	**44.7**
	Two plants/hill	1.57	1.83	1.68	1.51	**1.64**	6.99	7.06	7.08	6.89	**7.00**	20.7	21.0	20.9	20.8	**20.8**	44.9	43.7	44.1	44.8	**44.3**
	Three plants/hill	1.67	1.95	1.82	1.61	**1.76**	7.45	7.65	7.60	7.43	**7.53**	21.1	21.6	21.4	21.2	**21.3**	44.5	43.4	43.8	44.5	**44.0**
Average of intercropping		**1.56**	**1.81**	**1.67**	**1.50**	**1.63**	**6.93**	**7.07**	**7.05**	**6.85**	**6.97**	**20.6**	**21.0**	**20.8**	**20.7**	**20.7**	**44.8**	**43.7**	**44.1**	**44.7**	**44.3**
Solid culture of soybean varieties		3.11	3.39	3.20	3.04	**3.18**	7.13	7.13	7.13	7.13	**7.13**	21.6	22.5	22.2	21.8	**22.0**	38.9	37.1	37.8	38.7	**38.1**
General mean of soybean varieties		**2.33**	**2.60**	**2.43**	**2.27**	**2.40**	--	--	--	--	--	**21.1**	**21.7**	**21.5**	**21.2**	**21.3**	**41.8**	**40.4**	**40.9**	**41.7**	**41.2**
L.S.D. at 5% of cropping systems (S)						0.95					N.S.					0.87					1.58
L.S.D. at 5% of soybean varieties (V)						0.28					N.S.					0.58					0.73
L.S.D. at 5% of distributions of corn plants (D)						0.25					0.56					0.43					0.49
L.S.D. at 5% of S x V						1.07					N.S.					1.02					1.71
L.S.D. at 5% of S x D						1.13					N.S.					1.14					1.98
L.S.D. at 5% of V x D						0.32					N.S.					0.71					0.91
L.S.D. at 5% of S x V x D						1.33					N.S.					1.38					2.17

Distributions of corn plants: Tripling number of corn plants from one to three plants per hill affected significantly plant height, seed yield per plant and per ha, seed oil and protein contents, whereas, seed index was not affected (Tables 4 and 5). Corn plant geometry which was distributed at wide distance (90 cm) increased seed yields per plant and per ha and seed oil content as compared to the other distributions. Increasing number of corn plants from one to three plants per hill increased seed yield per plant and per ha by 19.5 and 17.3 per cent, respectively. These results may be due to decrease in inter-specific competition between corn and soybean plants under the highest distribution of corn plants.

Soybean vegetative growth parameters performed better at 90 cm corn hill spacing than the other distances (Table 3). Soybean plants were more efficient in utilizing solar energy and consequently more dry matter accumulation in different parts of soybean plant organs under the highest distribution of corn plants (90 cm). Increasing number of corn plants from one to three plants per hill contributed mainly in a good configuration of the attributes of the soybean crop.

Shade treatments consistently decreased both protein and oil content per soybean seed when compared to the control, resulting in increased seed protein concentration and decreased seed oil concentration (Proulx and Naeve, 2007). These results are in agreement with those reported by Mazour (2011) who mentioned that intercropped soybeans with corn showed a consistent yield decline in the one row adjacent to corn regardless of corn population configuration. Also, Metwally *et al.* (2012) indicated that there are significant increments in seed yield per plant and per ha and seed oil content by increasing distance between hills of corn plants from 30 to 60 cm.

Interactions among cropping systems, soybean varieties and distributions of corn plants: With respect to response of soybean varieties to cropping systems, plant height, seed yields per plant and per ha, seed oil and protein contents reached the 5 per cent level of significance, whereas, seed index did not reach the same level of significance (Tables 4 and 5). Solid planting of soybean variety Giza 22 recorded higher values of seed yields per plant and per ha and seed oil content, whereas, the lower values of seed yields per plant and per ha were obtained by intercropping corn with soybean variety Giza 111. Also, the lowest seed oil content was obtained by intercropping corn with soybean variety Giza 21. Similar results were reported by Sharma and Mehta (1988) who found that cropping system x soybean variety interaction was significant for seed yield per plant but there were insignificant interactions for seed index, seed oil and protein contents. Also, Mudita *et al.* (2008) indicated that soybean cultivar by cropping system interaction was significant interaction for soybean yield per ha in one year. Moreover, Egbe (2010) revealed that intercropping significantly depressed yield of TGX 536-O2D soybean variety but the yield reductions in the other two soybean varieties were not significant.

With respect to response of distributions of corn plants to cropping systems, plant height, seed yields per plant and per ha, seed oil and protein contents reached the 5 per cent level of significance, whereas, seed index did not reach the same level of significance (Tables 4 and 5). Solid planting of soybean recorded higher values of seed yield per plant and per ha and seed oil content, while, the lower values of seed yield per plant and per ha and seed oil content were obtained by intercropping soybean with corn grown at narrow distance between hills (30 cm). These results are confirmed with those obtained by Abou-Elela *et al.* (2012) who showed that seed yield per plant and per unit area was affected significantly by the interaction between cropping systems and distributions of corn plants.

With respect to response of soybean varieties to distributions of corn plants, plant height, seed yield per plant and per ha, seed oil and protein contents reached the 5 per cent level of significance, whereas, seed index did not reach the same level of significance (Tables 4 and 5). Intercropping soybean variety Giza 22 with corn which grown at wide distance between hills (90 cm) recorded higher values of seed yield per plant and per ha and seed oil content, while, the shortest plants were obtained by intercropping soybean variety Giza 35 with corn which grown at narrow distance between hills (30 cm). Intercropping soybean variety Giza 111 with corn which grown at one plant per hill produced lower values of seed yield per plant and per ha and seed oil content. These results are in agreement with those reported by Muoneke *et al.* (2007) who found that seed index and pod production of soybean varieties were not affected by the interaction between corn plant density and soybean varieties in both seasons.

With respect to response of cropping systems and soybean varieties to distributions of corn plants, plant height, seed yield per plant and per ha, seed oil and protein contents reached the 5 per cent level of significance, whereas, seed index did not reach the same level of significance (Tables 4 and 5). Solid planting of soybean variety Giza 22 recorded higher values of seed yield per plant and per ha and seed oil content, whereas, the lower values of seed yield per plant and per ha were obtained by intercropping soybean variety Giza 111 with corn grown at 30 cm hill spacing. Moreover, intercropping soybean variety Giza 21 with corn which grown at wide distance (90 cm) produced the tallest plants. These results are in a good line with those obtained by Muoneke *et al.* (2007) who mentioned that there was no effect of corn plant density x cropping system x soybean variety on seed index of soybean plant.

Competitive relationships

Land equivalent ratio (LER): In general, intercropping culture increased LER as compared to solid plantings of corn and soybean (Fig. 2). It ranged from 1.32 by intercropping corn at 30 cm hill spacing with soybean variety Giza 111 to 1.64 by intercropping corn at wide distance between hills (90 cm) with soybean variety

Giza 22. Intercropping corn with soybean variety Giza 22 under 90 cm hill spacing gave an increase of LER over those obtained by the other treatments. This advantage of the highest LER over the others was due to the ratio of area occupied by soybean and corn which were 100 per cent of soybean for intercropping culture as compared with solid planting of soybean and 100 per cent of corn for intercropping culture as compared with solid planting of corn.

These data indicated that soybean plants are less able to use the available environmental resources through the stages of the soybean vegetative growth compared to corn plant under intercropping conditions (Table 2). Similar observations were reported by Hiebsch *et al.* (1995) who grew corn at 12 densities (from 1.0 to 10.0 plants/m^2) and two soybean cultivars, representing Maturity Groups VIII (Cobb) and VI (Davis), at three densities (3.0, 8.5, and 24.0 plants/m^2) in intercrop and sole crop in fan designs. They reported that Cobb -corn intercrop at 24 soybean plants per m^2 over a range of corn densities utilized the long growing season more productively (LER = 1.4) than in sole crop.

Also, Metwally *et al.* (2009) found that intercropping cultures increased LER as compared to solid plantings of corn and soybean. Moreover, Egbe (2010) showed that all intercrop combinations had LER above unity at the densities of soybean tested.

Land equivalent coefficient (LEC): LEC is a measure of interaction concerned with the

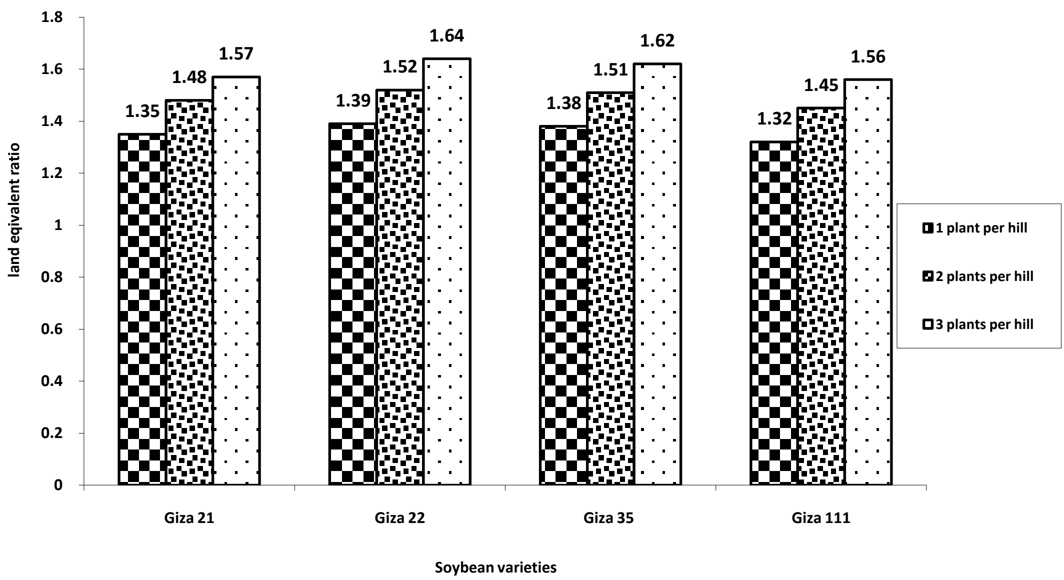


Figure 2. Land equivalent ratio (LER) as affected by cropping systems, soybean varieties, distributions of corn plants and their interactions (combined data across 2011 and 2012 seasons)

strength of relationship. LEC is used for a two- crop mixture the minimum expected productivity coefficient (PC) is 25 per cent, that is, a yield advantage is obtained if LEC value was exceeded 0.25. The effects of cropping systems, soybean varieties and their interactions on the LEC of intercropped corn with soybean were exceeded 0.25 under the intercropping culture (Fig. 3). Mean LEC values varied from 0.39 by growing corn in narrow distance between hills (30 cm) with soybean variety Giza 111 to 0.60 by growing corn in wide distance between hills (90 cm) with soybean variety Giza 22. Similar results were reported by Egbe (2010) who demonstrated that mean LEC values varied from 0.78 to 0.98 under treatments of study.

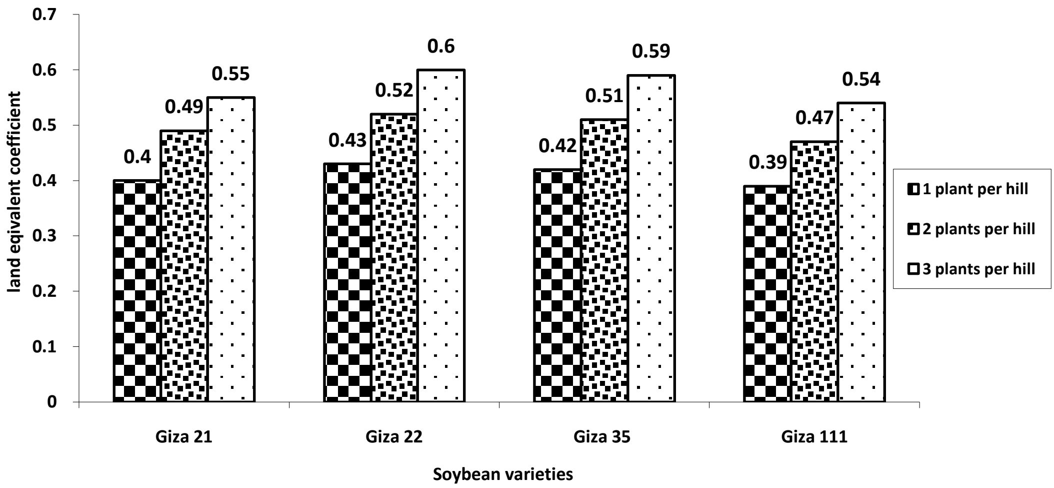
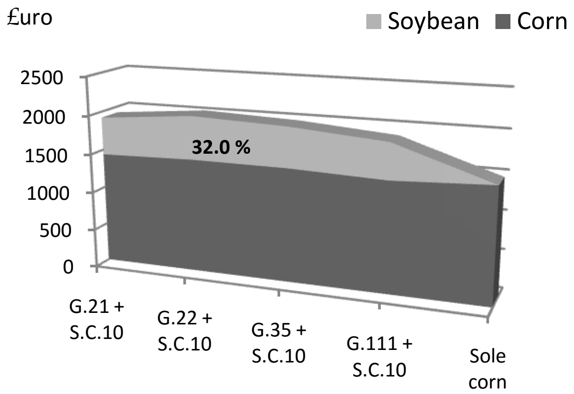


Figure 3. Land equivalent coefficient (LEC) as affected by cropping systems, soybean varieties, distributions of corn plants and their interactions (combined data across 2011 and 2012 seasons)

Economic evaluation

Mixed intercropping pattern increased total and net returns by about 32.0 and 27.3 per cent, respectively, as compared with solid planting of corn (Fig. 4 and Table 6). Net return of intercropping corn with soybean was varied between treatments from £uro 434 to 576 per ha as compared with solid planting of corn (£uro 395). Intercropping soybean variety Giza 22 with corn which distributed in three plants per hill gave the highest financial value when using high population densities of both crops and distributing corn plants at a wide distance between hills (90 cm).

The study suggested that intercropping soybean with corn is more profitable to farmers than solid planting of corn provided farmers use suitable intercropping pattern. These findings are parallel with those obtained by Asmat *et al.* (2007) and Metwally *et al.* (2009) who mentioned that all intercropping systems gave substantially higher net income over mono- cropping with higher net income Moreover, Egbe (2010) reported that soybean intercropped with sorghum at 333,000



Figgure 4. Total return as affected by cropping systems, soybean varieties, distributions of corn plants and their interactions (combined data across 2011 and 2012 season)

plants per ha gave higher net benefits and marginal rate of return than those planted at 200,000 and 400,000 plants per ha.

It could be concluded that although mixed intercropping pattern resulted in adverse effects on soybean yield and its attributes, however, Egyptian farmers could achieve an increase in their income by 86.8 per cent as compared to solid culture of corn when growing soybean plants (Giza 22) on both sides of corn ridges which distanced at 90 cm between corn hills. This paper suggest that intercropping pattern, soybean and corn varieties, distributions of corn plants and other cultural practices such as fertilizers and irrigation should be further investigated to improve the efficiency of intercropping corn with soybean plants.

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Table 6. Financial returns from cropping systems, soybean varieties, corn plant distributions and their interactions (combined data across 2011 and 2012 seasons)

Parameters/ Cropping systems	Financial returns per ha (£uro)							
	Corn				Soybean			
	Distributions of corn plants			Mean	Distributions of corn plants			Mean
	1 plant/ hill	2 plants/ hill	3 plants/ hill		1 plant/ hill	2 plants/ hill	3 plants/ hill	
Soybean varieties								
Giza 21	1321	1449	1546	**1439**	449	486	515	**484**
Giza 22	1352	1464	1587	**1468**	513	565	602	**560**
Giza 35	1346	1469	1577	**1464**	473	521	562	**519**
Giza 111	1295	1428	1541	**1422**	426	468	497	**464**
Mean	**1328**	**1453**	**1563**	**1448**	**466**	**510**	**544**	**506**
Solid planting of corn				**1480**				**---**
Solid planting of soybean (Giza 21)				**---**				**961**
Solid planting of soybean (Giza 22)				**---**				**1046**
Solid planting of soybean (Giza 35)				**---**				**987**
Solid planting of soybean (Giza 111)				**---**				**937**

	Total				Net			
	Distributions of corn plants			Mean	Distributions of corn plants			Mean
	1 plant/ hill	2 plants/ hill	3 plants/ hill		1 plant/ hill	2 plants/ hill	3 plants/ hill	
Soybean varieties								
Giza 21	1770	1935	2062	**1922**	319	484	610	**471**
Giza 22	1865	2029	2189	**2027**	414	578	738	**576**
Giza 35	1820	1990	2139	**1983**	369	539	688	**532**
Giza 111	1722	1896	2038	**1885**	270	445	587	**434**
Mean	**1794**	**1962**	**2107**	**1954**	**343**	**511**	**656**	**503**
Solid planting of corn				**1480**				**395**
Solid planting of soybean (Giza 21)				**961**				**126**
Solid planting of soybean (Giza 22)				**1046**				**211**
Solid planting of soybean (Giza 35)				**987**				**152**
Solid planting of soybean (Giza 111)				**937**				**102**

Prices of main products are that of 2011: £uro 207.3 for ton of corn; £uro 308.1 for ton of soybean; intercropping corn with soybean increased variable costs of intercropping culture from £uro 39 – 181 over those of solid planting of corn.

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Root Dynamics in Soybean (*Glycine max* L.) under Two Moisture Regimes

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ABSTRACT

Seven popular genotypes were grown in PVC pipes to study root morphological traits under two moisture regimes, well watered (WW) and low moisture stress (LMS) conditions. Root study revealed that mean root length under LMS condition was significantly (26 %) increased compared to WW condition. But root to shoot dry weight ratio did not vary much between WW and LMS conditions. Maximum phenotypic (PCV) and genotypic (GCV) coefficient of variation were observed for root volume, root fresh weight and root dry weight under WW condition. Under LMS condition, PCV was maximum for root to shoot dry weight ratio followed by root volume, which had maximum GCV. Root volume recorded high heritability coupled with high genetic advance under both WW and LMS conditions along with root fresh weight and root dry weight under WW and number of leaves and root fresh weight under LMS condition, indicating the reliability of these traits for selection to isolate drought resistant genotypes.

Key words: moisture stress, root morphology, soybean

Drought resistance is a complex trait that depends on action and reaction of different phenological, morphological, physiological and biochemical characters. Among root morpho-logical characters, root system has been considered as an important component of drought resistance and determines yield (Passioura, 1982; Thanh *et al.*, 1999). Among various root morphological traits, maximum root length was reported to be associated with drought tolerance. Plants having deeper root system will colonize a large soil volume and improve water uptake from the lower layers where, water is expected to be available. This would help to maintain a good plant water potential which has demonstrated positive effect on yield under stress (Mumbani and Lal, 1983).

Since, root traits are invisible (under natural growth conditions), it is

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practically difficult to screen large germplasm and segregants from crossing programmes for root morphological traits. So, the information on relationship between plant type and root growth would facilitate the breeder to select for root traits based on above ground traits (Yoshida *et al.*, 1982). Models for predicting various plant organs developed in angiosperm and conifer tree species have been used in predicting biomass of one organ based on another organ (Niklas and Enquist, 2002).

Root modelling may be helpful in bypassing the tedious root sampling in root screening studies as it will be helpful in predicting the root traits based on above ground traits. The root modelling was attempted and successfully done in one or two crops like rice and sorghum. Since all the crops suffer from water stress in one or the other stage of growth period, there is a need to conduct the experiments on root modelling in variety of crops. In the light of above facts, the experiment was conducted to document the variability for root morphological traits of soybean under two moisture regimes, well watered (WW) and low moisture stress (LMS).

MATERIAL AND METHODS

Seven genotypes (Hardee, KHSb 2, KB 79, MACS 450, MAUS 2, MRSB 345 and NRC 67) were used for the present investigation. These were supplied by Soybean Scheme, UAS, GKVK, Bangalore.

The experiment was carried out at University of Agricultural Sciences, Bangalore, adopting completely randomized design (CRD). Two treatments of different irrigation levels, well watered (WW) and low moisture stress (LMS) were given. Each treatment was replicated thrice. These genotypes were raised in poly venyl chloride (PVC) pipes (100 cm long and 18 cm diameter). All the pipes were filled with top soil and compacted by using soil compactor leaving 15 cm at the top to fill with mixture of sandy loam and clay soil and FYM in 4:1 proportion. Four to five seeds of each entry were hand dibbled. After germination one healthy seedling was allowed to grow in each pipe. The soil in each pipe was fertilized according to recommended package of practices. Watering was done at four days interval for well watered (WW) and at 8 days interval for low moisture stress (LMS) condition.

Root sampling was done at 85 days old crop after recording observations on shoot morpho-logical characters and later on removing shoot portion of the plants. The PVC pipes were soaked overnight in standing water to loosen the soil. The next day, roots were separated from pipes and washed thoroughly by taking at most care not to damage roots and the observations were recorded on root traits (Fig 1 and 2).

RESULTS AND DISCUSSION

Analysis of variance for all the characters studied under both well watered (WW) and low moisture stress (LMS) conditions (Table 1) revealed significant differences among the genotypes for almost all characters studied except root length to shoot length ratio and root to shoot dry weight ratio both under WW and LMS conditions. Combined analysis of variance results showed

Fig. 1. Pictorial representation of root sampling

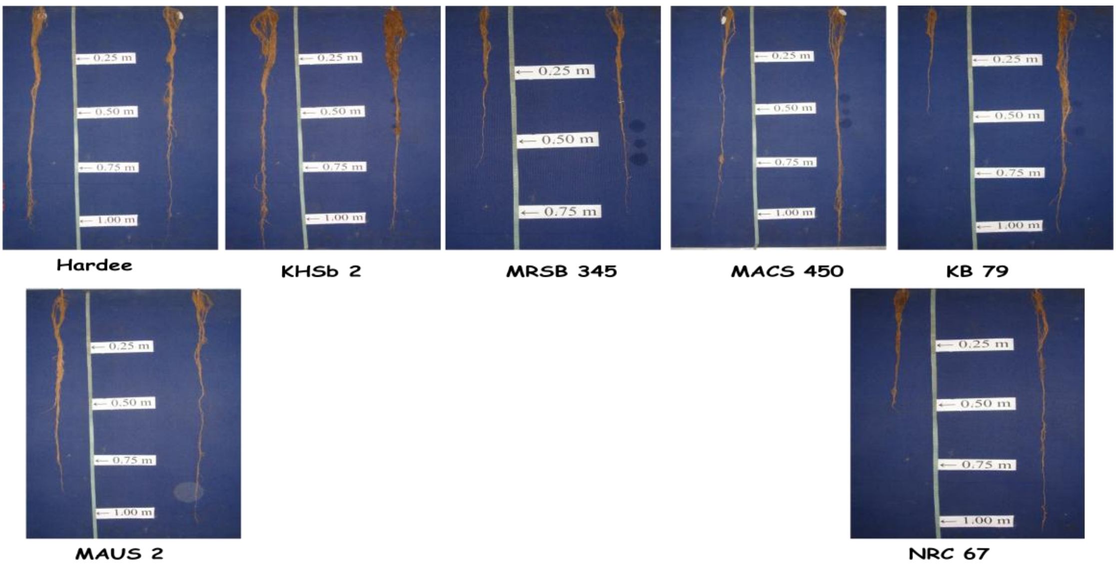

Fig. 2. **Soybean roots under WW (left) and LMS (right) conditions**

significant interaction for almost all the traits except root length to shoot length and root to shoot dry weight ratio. The mean values for the characters studied are furnished in table 2 for WW and LMS conditions, respectively.

Different genetic parameters studied, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (h^2) and genetic advance as per cent mean (GA) for all traits are furnished in table 3 for WW and LMS conditions.

The mean root length under WW condition was 80.05 cm with the highest value of 121.33 cm (KHSb 2) and lowest of 47.67 cm (KB 79), whereas under LMS condition the mean value recorded was 101.33 cm with highest length of 117.33 cm (MACS 450) and the lowest length of 74.33 cm (MRSB 345). Under WW condition, this trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean. GCV, PCV, h^2 and GA as per cent mean values were 37.57, 37.97, 97.89 and 76.57, respectively. Observed moderate GCV, moderate PCV, high h^2 and high GA as per cent mean under LMS condition. GCV, PCV, h^2 and GA as per cent mean values were 12.88, 13.17, 95.62 and 25.95, respectively.

Mean root volume was 9.29 cc and 6.57 cc under WW and LMS conditions, respectively. Highest volume of 17.33 cc was observed for Hardee and KHSb 2 under WW condition whereas MACS 450 recorded highest volume under LMS condition (11.67 cc). Lowest volume of 2.33 cc 2.00 cc was observed for MRSB 345 under WW and LMS conditions, respectively. This trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean under WW condition. GCV, PCV, h^2 and GA as per cent mean values were 63.60, 66.74, 90.82 and 124.86, respectively. Under LMS this trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean. Mean values of GCV, PCV, h2 and GA as per cent mean were 54.20, 56.79, 91.11 and 106.58, respectively.

KHSb 2 and NRC 67 were observed with highest (25.00 g) and lowest (4.33 g) root fresh weights respectively under WW condition, here as MACS 450 (14.33 g) recorded highest and NRC 67 (3.67 g) lowest root fresh weights under LMS condition. The mean value for the trait was 12.86 g and 8.71 g under WW and LMS conditions, respectively. The trait exhibited high GCV, PCV, h^2 and GA as per cent mean under WW condition. GCV, PCV, h^2 and GA as per cent mean values were 63.34, 66.21, 91.52 and 124.82, respectively. Under LMS condition, it showed high GCV, PCV, h^2 and GA as per cent mean with corresponding values of 42.64, 45.89, 86.30 and 81.59, respectively.

Root dry weight was maximum for the genotype KHSb 2 (6.67 g) and minimum for MRSB 345 (1.00 g) with the mean value of 3.29 g under WW condition, whereas under LMS condition, maximum root dry weight (4.67g) was observed for KHSb 2 and MACS 450. The lowest value (1.67 g) was observed for MRSB 345 and NRC 67. Under WW condition, the trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean. GCV, PCV, h^2 and GA as per cent mean values were 63.74, 68.41, 86.81 and 122.34, respectively.

Table 1. Analysis of variance for eleven characters under WW and LMS condition

Source of Variation	Deg-rees of free-dom	Root length	Root volume	Root fresh weight	Root dry weight	Shoot length	Numb er of leaves	Shoot fresh weight	Shoot dry weight	Plant length	Root length to shoot length ratio	Root to shoot dry weight ratio
						WW condition						
Genotype	6	2732.60**	108.16**	205.10**	13.83**	567.75**	955.32**	210.86**	66.21**	5205.97**	0.581	0.041
Error	14	19.52	3.52	6.14	0.67	5.95	19.62	30.71	5.14	22.1	0.037	0.0051
CV (%)		5.52	20.22	19.28	24.85	5.43	11.11	14.75	14.34	3.77	10.8	34.49
						LMS condition						
Genotype	6	518.89**	39.30**	43.60**	5.30**	143.75**	555.76**	192.56**	58.49**	897.52**	0.271	0.0376
Error	14	7.81	1.24	2.19	1	3.67	3.76	5.67	4.52	10.71	0.009	0.0083
CV (%)		12.76	16.93	16.98	30.88	13.9	5.47	7.07	12.01	12.18	4.57	45.19

*** Significant at 0.01 level*

Table 2. Mean values for the eleven characters studied under WW and LMS conditions

Genotypes/ Traits	Root length (cm)	Root volume (cc)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Number of leaves	Shoot fresh weight (g)	Shoot dry weight (g)	Plant length (cm)	Root length to shoot length ratio	Root to shoot dry weight ratio
WW condition											
Hardee	102.00	17.33	23.33	5.67	61.67	58.33	44.67	20.33	160.00	1.65	0.28
KHSb 2	121.33	17.33	25.00	6.67	66.00	64.67	50.33	16.33	189.33	1.84	0.41
MRSB 345	53.00	2.33	6.33	1.00	31.33	21.00	28.33	8.33	84.33	1.70	0.12
MACS450	103.33	7.67	9.67	3.00	46.00	51.67	34.67	12.33	146.33	2.25	0.26
KB 79	47.67	4.33	7.67	1.67	38.67	25.00	29.33	13.67	88.33	1.24	0.12
MAUS 2	83.67	10.00	13.67	3.33	34.33	31.33	42.67	21.67	118.00	2.45	0.15
NRC 67	49.33	6.00	4.33	1.67	36.33	27.00	33.00	18.00	85.67	1.37	0.10
Mean	**80.05**	**9.29**	**12.86**	**3.29**	**44.90**	**39.86**	**37.57**	**15.81**	**124.57**	**1.79**	**0.21**
SEm (±)	1.30	1.29	1.77	0.47	1.88	1.78	1.01	1.06	1.67	0.10	0.03
CD (0.05)	**30.18**	**6.00**	**8.27**	**2.15**	**13.76**	**17.85**	**8.38**	**4.70**	**41.66**	**0.44**	**0.11**
LMS conditions											
Hardee	108.00	10.67	12.33	4.00	58.33	61.00	39.67	21.33	166.33	1.86	0.19
KHSb 2	104.00	7.33	9.00	4.67	59.00	47.67	44.67	22.33	163.00	1.76	0.21
MRSB 345	74.33	2.00	4.67	1.67	43.67	27.67	28.33	17.33	118.00	1.70	0.10
MACS450	117.33	11.67	14.33	4.67	49.67	27.67	28.33	12.00	167.00	2.36	0.41
KB 79	102.33	5.67	8.00	2.33	43.00	26.00	28.00	14.33	145.33	2.38	0.16
MAUS 2	101.67	5.67	9.00	3.67	46.33	32.33	25.00	14.00	148.00	2.19	0.26
NRC 67	101.67	3.00	3.67	1.67	43.67	26.00	41.67	22.67	145.33	2.33	0.08
Mean	**101.33**	**6.57**	**8.71**	**3.24**	**49.10**	**35.48**	**33.67**	**17.71**	**150.43**	**2.09**	**0.20**
SEm (±)	1.77	0.78	0.83	0.33	1.48	1.84	1.71	0.99	1.63	0.06	0.03
CD (0.05)	**13.15**	**3.62**	**3.81**	**1.33**	**6.92**	**13.61**	**8.01**	**4.42**	**17.30**	**0.30**	**0.11**

Table 3. Genetic variability parameters for eleven traits under WW and LMS conditions

Trait	Mean	Minimum	Maximum	GCV (%)	PCV (%)	Heritability (%)	GA as % mean
			WW condition				
Root length (cm)	80.05	47.67	121.33	37.57	37.97	97.89	76.57
Root volume (cc)	9.29	2.33	17.33	63.60	66.74	90.82	124.86
Root fresh weight (g)	12.86	4.33	25.00	63.34	66.21	91.52	124.82
Root dry weight (g)	3.29	1.00	6.67	63.74	68.41	86.81	122.34
Shoot length (cm)	44.90	31.33	66.00	30.47	30.95	96.92	61.80
Number of leaves	39.86	21.00	64.67	44.31	45.68	94.08	88.54
Shoot fresh weight (g)	37.57	28.33	50.33	20.62	25.36	66.16	34.56
Shoot dry weight (g)	15.81	8.33	21.67	28.54	31.94	79.83	52.52
Plant length (cm)	124.57	84.33	189.33	33.37	33.58	98.74	68.31
Root length to shoot length ratio	1.79	1.24	2.45	23.83	26.16	82.96	44.71
Root to shoot dry weight ratio	0.21	0.10	0.41	53.07	63.29	70.31	91.67
			LMS condition				
Root length (cm)	101.33	74.33	117.33	12.88	13.17	95.62	25.95
Root volume (cc)	6.57	2.00	11.67	54.20	56.79	91.11	106.58
Root fresh weight (g)	8.71	3.67	14.33	42.64	45.89	86.30	81.59
Root dry weight (g)	3.24	1.67	4.67	36.98	48.18	58.91	58.47
Shoot length (cm)	49.10	43.00	59.00	13.92	14.45	92.72	27.61
Number of leaves	35.48	26.00	61.00	38.24	38.62	98.00	77.97
Shoot fresh weight (g)	33.67	25.00	44.67	23.44	24.49	91.66	46.24
Shoot dry weight (g)	17.71	12.00	22.67	23.94	26.79	79.91	44.09
Plant length (cm)	150.43	118.00	167.00	11.43	11.63	96.50	23.13
Root length to shoot length ratio	2.09	1.70	2.38	14.16	14.88	90.58	27.76
Root to shoot dry weight ratio	0.20	0.08	0.41	48.90	66.58	53.94	73.98

This trait exhibited high GCV, high PCV, medium h2 and high GA as per cent mean under LMS. Mean values of GCV, PCV, h^2 and GA as per cent mean were 36.98, 48.18, 58.91and 58.47, respectively.

The highest shoot length of 66 cm and 59 cm was recorded for KHSb 2 under WW and LMS conditions, respectively. The lowest of 31.33 cm (MRSB 345) and 43.00 cm (KB 79) was observed under WW and LMS conditions, respectively. The mean value of the trait was 44.90 cm (WW) and 49.10 cm (LMS). Under WW condition, this trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean. GCV, PCV, h^2 and GA as per cent mean values were 30.47, 30.95, 96.92 and 61.80, respectively. This trait exhibited moderate GCV, moderate PCV, high h^2 and high GA as per cent mean under LMS condition. Mean values of GCV, PCV, h^2 and GA as per cent mean were 13.92, 14.45, 92.72 and 27.61, respectively.

The number of leaves was highest (64.67) in KHSb 2 and lowest (31.33) in MRSB 345 with the mean value of 39.86 under WW condition. Similarly under LMS condition, Hardee recorded highest number of leaves (61.00) and KB 79 and NRC 67 recorded lowest number of leaves (26.00); average being 35.48. Under WW condition, this trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean. Mean values of GCV, PCV, h^2 and GA as per cent mean were 44.31, 45.68, 94.08 and 88.54, respectively. Under LMS condition, this trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean. Mean values of GCV, PCV, h^2 and GA as per cent mean were 38.24, 38.62, 98.00 and 77.97, respectively.

Under WW condition, the mean shoot fresh weight was 37.57 g with the maximum of 50.33 g (KHSb 2) and minimum of 28.33 g (MRSB 345), whereas the mean value of the trait under LMS condition was 33.67 g. KHSb 2 recorded highest (44.67 g) and MAUS 2 recorded lowest (25.00 g) shoot fresh weight. Under WW condition, this trait exhibited high GCV, PCV, h^2 and GA as per cent mean. GCV, PCV, h^2 and GA as per cent mean values were 20.62, 25.36, 66.16 and 34.56, respectively. Under LMS condition, it showed high GCV, PCV, h^2 and GA as per cent mean with corresponding values of 23.44, 24.49, 91.66 and 46.24, respectively.

The mean shoot dry weight under WW condition was 15.81 g, ranging from 8.33 g (MRSB 345) to 21.67 g (MAUS 2). The range under LMS condition was between 22.67 g (NRC 67) and 12.00 g (MACS 450) with the mean value of 17.71 g. Under WW condition this trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean with corresponding values of 28.54, 31.94, 79.83 and 52.52, respectively. Under LMS condition, this trait exhibited moderate GCV, moderate PCV, moderate h^2 and high GA as per cent mean with corresponding values of 23.94, 26.79, 79.91 and 44.09, respectively.

The mean plant length under WW condition was 124.57 cm ranging from 84.33 cm (MRSB 345) to 189.33 cm (KHSb 2). Under LMS condition, Hardee recorded highest (166.33 cm) and MRSB 345 recorded lowest 1(18.00 cm) plant length. This trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean under WW condition. GCV, PCV, h^2 and GA as per cent mean values were 33.37, 33.58, 98.74 and 68.31, respectively. Under LMS condition, moderate GCV, moderate PCV, high h^2 and high GA as per cent mean with corresponding values of 11.43, 11.63, 96.50 and 23.13, respectively.

Under WW condition the mean root to shoot length ratio was 1.79, ranging between 1.24 (KB 79) and 2.45 (MAUS 2). The range for the trait under LMS condition was between 1.70 (MRSB 345) and 2.38 (KB 79) with the mean value of 2.09. This trait exhibited high GCV, PCV, h^2 and GA as per cent mean under WW condition with corresponding values of 23.83, 26.16, 82.96 and 44.71, respectively. Under LMS condition, moderate GCV, PCV, high h^2 and high GA as per cent mean. GCV, PCV, h^2 and GA as per cent mean values were 14.16, 14.88, 90.58 and 27.76, respectively.

The mean root to shoot dry weight ratio under WW condition was 0.21, ranging from 0.10 (NRC 67) to 0.41 (KHSb 2), whereas under LMS condition the ratio was 0.20, ranging from 0.08 (NRC 67) to 0.41 (MACS 450). Under WW condition, this trait exhibited high GCV, high PCV, high h^2 and high GA as per cent mean. GCV, PCV, h^2 and GA as per cent mean values were 53.07, 63.29, 70.31and 91.67, respectively. Under LMS condition, it showed high GCV, high PCV, moderate h^2 and high GA as per cent mean with corresponding values of 48.90, 66.58, 53.94 and 73.98, respectively.

Among the seven genotypes, KHSb 2 was found to be deep rooted under WW condition while MACS 450 under LMS condition. MRSB 345 and Hardee under WW condition and MACS 450 under LMS condition found to have maximum root volume. MAUS 2 and KB 79 found to have maximum root length to shoot length ratio under WW and LMS conditions, respectively. Root to shoot dry weight ratio was maximum for KHSb 2 under WW condition and for MACS 450 under LMS condition. Soybean genotypes showed high GCV and PCV values for all the traits studied under WW condition while under LMS except for shoot length, root length, root length to shoot length ratio, other traits exhibited high GCV and PCV values indicating high variability among the genotypes. Except root dry weight and root to shoot dry weight ratio under LMS all traits showed high heritability coupled with high GA as per cent of mean. These results were in accordance with Shashidhar (1990), Gomathinayagam *et al.* (1990); Latha (1996) Hemamalini (1997), Kanbar (2001) and Prabudda (2003) in rice. There was significant interaction effects were observed for most of the traits except traits like root length to shoot length ratio and root to shoot dry weight ratio.

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Effect of Irrigation Levels on Yield and Water Productivity of Soybean under Vertisols

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ABSTRACT

A field experiment was conducted at Kota during kharif *2009 and 2010 on a clay loam soil to find out the influence of irrigation levels on critical stages for increasing the productivity and water use efficiency of soybean. Results revealed that irrigation applied at flower initiation + seed filling (20 days after flower initiation) stage recorded significantly higher dry matter accumulation at 30, 45 and 60 DAS and CGR at 30-45 and 45-60 days after sowing (DAS) as compared to control. Maximum and significantly higher plant height, branches per plant, pods per plant, seeds per pod, seed index, seed yield (1,915 kg/ha), straw yield (2,762 kg/ha), net return (23,990 ₹/ha), B:C ratio (2.63) and water use efficiency (44.0 kg/ha-cm) was recorded with irrigation at flower initiation + seed filling stage, however, it remained statistically on par with irrigation at seedling (15-20 DAS) + flower initiation + seed filling stages with each other.*

Key word: Crop growth rate, economics, relative growth rate, soybean, water use efficiency

Soybean [*Glycine max* (L.) Merrill] is one of the important rainfed leguminous oilseed crops of central India. The uncertain on-set of monsoon and its non-uniform distribution over crop growth period is one among the major limiting factors for optimizing the productivity of soybean in Rajasthan. The aberration in temporal and spatial distribution makes the crop vulnerable to moisture stress/drought. The moisture stress at critical growth stages often leads to its lower productivity. Water stress decreased the ratio of main stem grain yield to total plant grain yield of soybean (Khadambashi *et al*., 1988). Sinaki *et al.* (2007) found that biological yield of soybean under moderate and severe drought stresses was decreased by 20.7 and 31.2 per cent, respectively over control. All the physiological processes of plant are directly and indirectly influenced by water status of plant. Water is essential

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for cell turgidity, which is related to photosynthesis, growth of cell tissues and organs (Reddi and Reddy, 1995). Timely irrigation enhanced the yield attributes and yield (Dhindwal *et al.*, 2006) while water stress at their critical stages reduced yield attributes and yield (Khadambashi *et al.*, 1988). Hence, present investigation was carried out to find out the influence of irrigation levels on critical stages for increasing the productivity and water use efficiency of soybean under Vertisols.

MATERIAL AND METHODS

Field experiments to find out the influence of irrigation levels on critical stages for increasing the productivity and water use efficiency of soybean were carried out at Agricultural Research Station, Kota (26^o North latitude, 76^o-6' East longitude and 260 m above mean sea level), Rajasthan during *kharif* 2009 and 2010. The soil of the experimental field belonged to Vertisols having bulk density 1.52 Mg per m^3, pH 7.77 and cation exchange capacity 35 Cmol per kg. The soil has a very low water intake rate 0.25 cm per h on surface but at deeper layer (1.2 to 1.5 m) it was impermeable. The potential moisture retention capacity of soil is 120 mm of water in 1 m depth. The soil of the experimental site was analyzed medium in organic carbon (5.6 g/kg), available nitrogen (320 kg/ha), available phosphate (23.0 kg P_2O_5/ha) and available potash (275 kg K_2O /ha). The study area falls under humid south eastern plain zone of Rajasthan. The total rainfall received during meteorological week (27 to 41) was 364.4 mm and 555.2 mm in 2009 and 2010, respectively (Table 1). The corresponding numbers of rainy days were 35 and 30, respectively. Irrigation requirement of the crop as per treatment was met out by ground water irrespective of rainfall. Supplemental irrigation was provided in case of less rainfall at scheduled irrigation treatment maintaining 6 cm depth of water during both the seasons. The experiment was laid out in randomized block design having 3 replications and net plot size of 2.4 m x 5.0 m. The experiment comprised of 8 irrigation treatments namely, irrigation at seedling stage (15-20 DAS), irrigation at flower initiation, irrigation at seed filling (20 days after flower initiation) stage, irrigation at seedling stage (15-20 DAS) + flower initiation stage, irrigation at seedling stage (15-20 DAS) + irrigation at seed filling (20 days after flower initiation) stage, irrigation at flower initiation + seed filling (20 days after flower initiation) stage, irrigation at seedling stage + flower initiation + seed filling stage and control (no irrigation). Normal crop of soybean (JS 97-52) was raised employing recommended package of practices. Soybean crop was sown on 7 and 12 July and harvested on 20 and 22 October in 2009 and 2010, respectively. Measurement of water at the time of full or supplemental irrigation was done by velocity area method. The water use efficiency was calculated on the basis of pod yield per cm water used by the soybean crop. Periodic dry matter accumulation per plant at 15 days interval was recorded. Samples were oven dried at 70^0 C for 72 h and weight was recorded. Crop growth rates (CGR) and relative growth rate (RGR) were calculated

Table 1. Weekly rainfall received during *kharif* 2009 and 2010

Standard week	Period	Total rainfall (mm)		Rainy days	
		2009	2010	2009	2010
27	July 2 to July 8	106.2	77.1	2	4
28	July 9 to July 15	33.4	11.6	4	1
29	July 16 to July 22	71.8	20.5	7	2
30	July 23 to July 29	11.0	32.8	0	2
31	July 30 to August 5	0.0	76.2	0	4
32	August 6 to August 12	5.6	48.1	1	5
33	August 13 to August 19	23.0	96.8	5	4
34	August 20 to August 26	5.4	116.0	2	4
35	August 27 to September 2	48.2	4.5	4	0
36	September 3 to September 9	2.0	31.8	4	3
37	September 10 to September 16	6.6	38.0	1	1
38	September 17 to September 23	0.0	1.8	0	0
39	September 24 to September 30	0.0	0	0	0
40	October 1 to October 7	33.2	0	5	0
41	October 8 to October 14 8	0.0	0	0	0
	Total	**364.4**	**555.2**	**35**	**30**

by standard procedure (Devasenapathy *et al.,* 2008) for different intervals. The economics was computed at prevailing market rate during both the years of study. The variation in net returns and B: C ratio among the treatments was due to irrigation charges @ Rs. 770 per ha per irrigation. The generated data pooled over two years was pooled and statistically analyzed for its test of significance employing standard procedures.

RESULTS AND DISCUSSION

Growth and physiological parameters

Dry matter production and physiological parameters differed significantly due to different irrigation treatments (Table 2). Maximum dry matter production at all the three stages of observations and plant height at harvest were recorded when the crop was irrigated at flower initiation + seed filling (20 days after flower initiation) stages which were at par with irrigation applied at seedling (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) stages. Dry matter production at 30, 45 and 60 DAS registered an increase of 17.07, 25.6 and 28.8 per cent over control (no irrigation), respectively. Data further revealed that irrigation applied at flower initiation and seed filling (20 days after flower initiation) stages have maximum and significantly higher crop growth rate (CGR) at 30-45 DAS (10.60 $g/m^2/day$) and at 45-60 DAS (19.82 $g/m^2/day$ but remained on par with irrigation at seedling (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) stages. The mean relative growth rate (RGR) recorded at 30-45 DAS and 45-60 DAS did not reveal any significant variations. These results are in accordance with the finding of Walia and Kler (2007) and Ali *et al*. (2009). Significantly higher number of branches per plant (4.20) was recorded with two irrigations applied at flower initiation + seed filling (20 days after flower initiation) stages over rest of the treatments except irrigation at seedling (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) stages and seedling (15-20 DAS) + seed filling (20 days after flower initiation) stages.

Yield attributes and yield

Significantly highest number of pods per plant (62.87) and seeds per pod (3.03) were obtained when irrigation applied at seedling (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) stages and flower initiation + seed filling (20 days after flower initiation) stages, respectively over control and other treatments except irrigations at seedling (15-20 DAS) + seed filling (20 days after flower initiation) stages (Table 3). The more number of pods per plant, seeds per pod and seed index under above treatment could be ascribed to better growth and accumulation of more photosynthetes as a result of adequate supply of water at critical stages of the crop. The results are in corroboration with the findings of Ali *et al.* (2009) and Pradhan *et al*. (2012). Whereas, higher seed index (8.71 g) was recorded when irrigation was applied at three different growth stages, namely seedling (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) stages over no irrigation (control), seedling, flower initiation and seedling + flower initiation stages, but remained statistically on par with flower initiation + seed filling, seedling + seed filling stages and seed filling stage. The reduction in seed index might be due to less development of seeds under stress conditions. These results are in accordance with the finding of Brown *et al*. (1985) and Ali *et al*. (2009).

Significantly maximum seed yield (1,925 kg/ha) and straw yield (2,784 kg/ha) were recorded when crop was irrigated at seedling stage (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) over control (no irrigation) and rest of the treatments except at par with irrigation at flower initiation + seed filling stages, seedling + seed filling and seed filling stages. The treatment flower initiation + seed filling stage recorded significantly higher seed and straw yields to the tune of 43.7 and 40.7 per cent over control. The difference in the seed yield among different irrigation treatments might be accounted for the differences in pods per plant, seeds per pod and seed index. These results are in line with the findings of Doss *et al.* (1974), Ali *et al.* (2009) and Pradhan *et al.* (2012). No significant influence was observed due to irrigation treatments on harvest index. These results corroborated the findings of Pradhan *et al.* (2012).

Economic evaluation

Significantly higher net returns (Rs

Table 2. Effect of irrigation levels on growth and physiological parameters of soybean (data pooled for two years)

Treatment	Plant height (cm)	Branches (No/ plant)	Dry Matter (g/plant) at			Mean CGR (g/m^2/day) at		Mean RGR (g/g/day) at	
			30 DAS	45 DAS	60 DAS	30-45 DAS	45-60 DAS	30-45 DAS	45-60 DAS
Irrigation at seedling (15-20 DAS) stage	68.2	3.64	2.49	6.17	13.07	8.17	15.32	0.0262	0.0217
Irrigation at flower initiation stage	71.6	3.50	2.68	6.72	14.70	8.99	17.70	0.0266	0.0225
Irrigation at seed filling (20 days after flower initiation) stages	72.2	3.93	2.70	6.87	15.30	9.26	18.73	0.0270	0.0231
Irrigation at seedling + flower initiation stages	73.0	3.57	2.69	6.75	14.79	9.00	17.85	0.0266	0.0226
Irrigation at seedling + seed filling stages	74.2	4.03	2.73	6.90	15.53	9.37	19.17	0.0271	0.0232
Irrigation at flower initiation + seed filling stages	77.8	4.20	2.88	7.65	16.57	10.60	19.82	0.0282	0.0222
Irrigation at seedling stage + flower initiation + seed filling stages	76.8	4.10	2.88	7.51	16.67	10.28	20.35	0.0278	0.0228
Control (no irrigation)	64.2	3.34	2.46	6.09	12.86	8.06	15.03	0.0261	0.0217
SEm (±)	2.57	0.145	0.052	0.240	0.441	0.422	0.451	0.0009	0.0014
CD at (P = 05)	**7.8**	**0.44**	**0.16**	**0.73**	**1.34**	**1.28**	**1.37**	**NS**	**NS**

Table 3. Effect of irrigation levels on yield, water use efficiency and economics of soybean (data pooled for two years)

Treatments	**Pods (No/ plant)**	**Seeds (No /pod**	**Seed index (g/100 seeds)**	**Seed yield (kg/ha)**	**Straw yield (kg/ha)**	**Harvest index (%)**	**Net returns (Rs/ha)**	**B:C ratio**	**WUE (kg/ha-cm)**
Irrigation at seedling (15-20 DAS) stage	42.17	2.53	8.05	1439	2114	40.59	15097	2.07	39.07
Irrigation at flower initiation stage	44.60	2.50	8.21	1433	2094	40.67	14951	2.07	38.00
Irrigation at seed filling (20 days after flower initiation) stages	53.67	2.80	8.42	1762	2577	40.77	21305	2.47	43.13
Irrigation at seedling + flower initiation stages	48.67	2.67	8.29	1556	2275	40.72	17087	2.19	36.02
Irrigation at seedling + seed filling stages	54.67	2.93	8.50	1799	2628	40.80	21656	2.45	41.94
Irrigation at flower initiation + seed filling stages	61.44	3.03	8.65	1915	2762	41.07	23990	2.63	44.00
Irrigation at seedling stage + flower initiation + seed filling stages	62.87	2.97	8.71	1925	2784	41.05	23817	2.56	42.00
Control (no irrigation)	39.30	2.33	8.03	1333	1963	40.49	13311	1.98	38.45
SEm (±)	2.72	0.079	0.128	60.7	77.1	0.27	1227.8	0.085	1.22
CD at (P = 05)	8.24	0.24	0.39	184	234	NS	3724	0.26	3.71

23,990/ha) and B:C (2.63) ratio were recorded with irrigation at flower initiation + seed filling stages closely followed by irrigation at seedling (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) stages and lowest net returns (Rs 13,311/ha) were recorded in control (Table 3). The magnitude of increased net returns was Rs 10,679 per ha over control (no irrigation). This might be due to no moisture stress at crop growth critical stages resulted higher yield. On the contrary in control, which experienced severe water stress resulted in lower values of yield attributes and lower yield. These results corroborate the findings of Ali *et al.* (2009).

Water use efficiency

Significantly maximum water use efficiency (WUE) (44.0 kg/ha-cm) was recorded in the treatment of irrigation applied at flower initiation + seed filling stages while minimum under the control (38.45 kg/ha-cm). However, it was found statistically on par with three irrigation treatments, namely seedling (15-20 DAS) + flower initiation + seed filling (20 days after flower initiation) stages, seedling + seed filling stages and seed filling stage (Table 3). It might be due to sufficient availability of moisture at critical crop growth stages. Higher WUE, perhaps owing to higher dry-matter production, which was reflected in higher physiological activity of the crop to exploit the supplied moisture efficiently, resulting in production of higher economic yield for every unit of water consumed by the crop. These results are in accordance with the finding of Pradhan *et al.* (2012).

Thus, study suggested that soybean is more sensitive to moisture stress during its flowering initiation and seed filling stage. The two irrigation at flowering initiation + seed filling (20 DAS after flower initiation) stages under long dry spell is essential to achieve the optimum yield and WUE.

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Effect of Purple Seed Stain Disease on Physical and Biochemical Traits of Soybean

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ABSTRACT

Healthy (asymptomatic) and various categories of purple stained seeds of cultivar NRC 7 were utilized to study influence of purple stain disease caused by Cercospora kikuchii *on physical and biochemical parameters of soybean seeds. Results indicated that* C. kikuchii *infected seeds exhibited marked reduction in germination and emergence when 25 per cent or more surface area of seed was stained. Post-emergence mortality was caused when more than 50 per cent of seed area was stained. Single to multiple cracks in seed coat were observed leading to prominent absence of hard seeds. Purple stain has significantly reduced test weight, seed and root vigour, normal seedlings, root and seedling length, speed of elongation and trifoliate leaves in seedlings, and increased dead seeds, abnormal seedlings and electrical conductivity. Loss in seed yield was in the range of 36-80 per cent. It also reduced stearic acid, palmitic acid, linoleic acid, linolenic acid, trypsin inhibitor and protein, and increased oleic acid as well as oil content in seeds of soybean.*

Key words: *Cercospora kikuchii*, emergence, germination, purple seed stain, soybean

Soybean [*Glycine max* (L.) Merrill] is a widely accepted crop for high protein and oil content and also for its nutraceutical and pharmaceutical use. Purple seed stain disease caused by *Cercospora kikuchii* (Matsumoto and Tomoyasu) Gardner, is one of the important diseases of soybean. This is considered to be the 5th most important yield–depressing disease in the world (Sinclair and Hartman, 1999). The purple seed stain caused yield loss of 19.12 lakh metric tonnes in top eight soybean producing countries in 2006 (Wrather *et al*., 2010). Disease also leads to reduced market grade on account of pink or a pale purple to a dark purple discolouration on seed coat, poor processing qualities, reduced seed

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vigour and delayed germination (Pathan *et al*., 1989; Jackson *et al*., 2006). As such, no precise information on the influence of degree of seed discolouration on physical characters of seeds, germination, vigour, biochemical traits and seedling characters is available in India. The present experiment was therefore, conducted to generate the information on these aspects.

MATERIAL AND METHODS

Healthy asymptomatic and different degrees of purple stained seeds of cultivar NRC 7 harvested from the rainy season of 2007, 2008 and 2009 were utilized for the study at Directorate of Soybean Research, Indore, India. The stained seeds were classified in following six categories based on the per cent seed coat area exhibiting purple stain: 1= 0, 2= 1 to 10, 3= 11 to 25, 4= 26 to 50, 5= 51 to 75 and 6= 76 to 100 per cent. Completely randomized block design for laboratory and net house experiments and randomized block design for field experiment was employed.

Observations were recorded on influence of different categories of purple stain on seed coat rupture, test weight and seed moisture content. For standard germination, paper towel method (ISTA, 1985) was followed. Observations were also made on the percentages of normal seedlings, abnormal seedlings, hard (un-imbibed) seeds and dead seeds. Speed of elongation was determined by measuring length of five seedlings from each category at 6th, 10th, and 14th day after germination utilizing formula of Carleton *et al*. (1968).

To measure the seed vigour, electrical conductivity of the seed leachate was measured at 24 h of soaking with conductivity meter model Systronic 306, following the procedure of Powel and Metthews (1992). The longevity of the seeds was tested by accelerated ageing test as described by Musgrave *et al.* (1980).

For estimation of oil content, seed samples of each category were ground and extracted with hexane in Socsplus for 3 h in triplicate. For estimation of protein content, nitrogen was determined through Kel plus kjeldhal unit and converted into protein content by multiplying with conversion factor (5.71). For trypsin inhibitor assay, standard procedure (Kakade *et al*., 1974) as modified by Hammerstrand *et al*. (1981) was followed. Fatty acid estimation was done by gas liquid chromatograph (GLC), Shimadzu GC 17A. The peaks for individual fatty acid methyl esters were identified by comparing the retention times with those of standard methyl esters (procured from Sigma Chemical Company). For determination of lipoxygenase isozymes (I and II+III) procedures of Marczy *et al.* (1995) and Axelrod *et al.* (1981) were followed. Total phenol was determined by the method of Singleton and Rossi (1965).

Under net house, one hundred seeds of categories 1 and 4 each were sown in 30 cm diameter earthenware pots with 10 seeds in every pot. Observations were taken on seedlings mortality, root length, shoot length, root vigour, number of trifoliate leaves and attachment of seed coat to cotyledons at 14 DAS. For root vigour visual score of 1 to 5 were assigned depending on the overall root biomass.

Purple stained seeds of different categories were also sown in a RBD with three replications on the experimental field with plot size of 5 m x 4 m. Observations were taken on field emergence and yield per plot (converted in kg/ha).

Data were subjected to an analysis of variance (ANOVA). Correlation studies and *t* test between stained seed categories and various seedling characters were also undertaken following Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Effect of purple stain on physical traits

Rupture of seed coat was observed in 4, 24, 24, 30, 36 and 76 per cent of seeds in categories 1, 2, 3, 4, 5 and 6, respectively. Cracks in seed coat were single in categories 1 and 2 while they were mostly multiple in other higher categories, indicating that purple seed infection led to cracking in seed coat which constantly increased with increase in infection category. The test weight (100 seed weight) was 17.2, 17.0, 17.1, 16.3, 15.2 and 14.0 g in seeds of categories 1, 2, 3, 4, 5, 6, respectively, reflecting deleterious influence of *C. kikuchii* on seed weight as also observed by Prasanth and Patil (2007).

Effect of purple stain on germination and seed viability

Germination rate was significantly and consistently reduced from a baseline of 86 per cent for asymptomatic seeds to 38 per cent for category 6 seeds, with intermediate germination rates in less severely stained seeds (Table 1). No hard seeds (non-imbibed) were observed in infected stained seeds. Though, effect of *C. kikuchii* on germination has been a matter of controversy (Sharma and Gupta, 2007) as, few workers (Pathan *et al*. 1989, Prasanth and Patil 2007) reported reduction in germination while other (Sediyama *et al*. 1971) did not associate it with reduced germination. Our data on germination indicated that *Cercospora kikuchii* infected seed led to marked reduction in germination only when more than 25 per cent surface area of seed coat got stained.

Correlation and 't' test studies between seed infection categories and various above-referred seed and seedling traits were also undertaken (Table 2). It revealed a positive correlation of seed infection category with seed coat rupture (p = 0.05) and negative with seedling length, test weight and speed of elongation (P = 0.01). However, 't' values were significant for test weight, seed coat rupture, germination, number of normal seedlings, number of dead seeds, seedling length and SE. It was also observed that vigour of the seeds of all stained seed categories was reduced with the increase in categories as revealed from accelerated ageing test (Table 3). Therefore, surviving plants produced from heavily infected seeds remained stunted and yielded low in the field experiment. Higher germination percentage and normal seedlings in category 1 and 2 seeds indicated that seeds of these categories were of high vigour and have better storability than seeds of infected categories of 3, 4, 5, and 6. Electrical conductivity (EC) consistently increased with the increase in seed infection category. It indicated that there

Table 1. Influence of purple seed infection on physical characteristics of seed

Seed infection category	Seed moisture (%)	Normal seedling (No)	Abnormal seedling (No)	Dead seed (No)	Hard seed (No)	Germination (%)	Seedling length* (cm)	Speed of elongation (cm/day)
1	5.3	79.3	6.6	7.3	6.7	86.0	23.2	0.095
2	5.0	79.4	7.4	13.4	0	79.4	21.7	0.095
3	4.0	69.3	11.3	19.3	0	69.3	21.7	0.096
4	5.0	56.0	16.7	27.3	0	56.0	21.3	0.094
5	4.7	53.3	17.3	29.3	0	53.3	20.4	0.086
6	5.7	37.7	9.4	53.4	0	37.7	18.9	0.083
C D (P = 0.05)	**0.7**	**8.2**	**3.3**	**8.3**	**0.7**	**9.0**	**3.9**	**-**

* *At 10 days after germination*

Table 2. Correlation coefficients (r) and "t" values of purple seed infection with various seed and seedling traits

	Test weight	Seed moisture	Seed coat rupture	Germination	Normal seedling	Abnormal Seedling	Dead seeds	Seedling Length	Seed elongation
"r"	**-0.97****	0.19	0.89*	-0.79	-0.77	0.24	0.77	-0.95**	-0.92**
"t"	**7.57****	0.38	3.90**	2.59*	2.41*	0.50	2.44*	6.13**	4.78**

*, **= *significant at 5 and 10%, respectively*

was disruption in membrane and therefore, it could not retain the solutes inside and allowed more and more solutes to leach out in the solution as the degree of seed infection increases. It also supports our finding that infected seeds do not have good storage potential. Emergence of seedlings from such seeds also remained low as it **is** commensurate with solute leakage.

Effect of purple seed stain on soybean growth and yield

In pot trial, purple seed infection significantly reduced germination, root length and shoot length, and enhanced post-emergence mortality to 9.0, 25.0, 23.0, 20.3 per cent, respectively (Table 4). Reduction in number of trifoliate leaves was 35.1 per cent. Root vigour was also reduced in seedlings developed from

Table 3. Influence of PSS infection on seed viability (accelerated ageing test) and electrical conductivity

Seed infection category	Germination (%)	Normal seedling (%)	Abnormal seedling (%)	Dead seed (%)	Hard seed (%)	Electrical Conductivity (ms) at 24 h
1	81.3	77.3	5.3	13.3	4	0.280
2	51.3	51.3	6.0	42.7	0	0.444
3	42.7	42.7	12.7	44.7	0	0.472
4	36.7	36.7	10.7	52.7	0	0.477
5	32.7	32.7	10	57.3	0	0.479
6	11.3	11.3	3.3	87.3	0	0.589
C D (P = 0.05)	**7.7**	**7.7**	**6.4**	**10.5**	**0.7**	

Table 4. Influence of PSS infection on germination and growth of seedlings (pot trial)

Characters	Healthy	Category 4 infected seeds	"t" value
Germination (%) (7DAS)	88	80	3.6*
POM (%)$	0	25	10.7***
Root Length (cm) $	14.8	11.4	3.2*
Shoot length (cm) $	30.6	24.4	4.3*
Leaves number$	1.54	1.0	1.4
Root vigour score (Av) $	4.8	3.3	2.5
With root vigour 5 (%)$	86.4	40	12.3***
Seed coat attached to cotyledons (%)$	4.76	20	26.1***

*$ Observations were taken 14 DAS; * Significant at 10%level; *** Significant at 1% level*

infected category 4. Seed infection of *C. kikuchii* thus led to increase in seedling mortality and marked reduction in seedling growth. Seed coat was also observed to remain attached to cotyledons in substantial number of seedlings developed from the category 4 seeds.

A perusal of table 5 reveals that field emergence of seedlings and yield was reduced consistently with the increase in purple stained category. The reduction in emergence was observed from baseline of 48.57 per cent in category 1 to 4.17 per cent of the category 6 seeds as well as

Table 5. Influence of purple seed stain on field emergence and yield

Seed infection category	Emergence (%)	Yield (kg/ha)
1	48.57	1473
2	35.14	937
3	17.22	859
4	17.00	609
5	9.58	365
6	4.17	295
C D (P = 0.05)	**4.6**	**205**

reduction in yield from baseline of 1,473 kg per ha in category 1 to 295 kg per ha of the category 6 seeds. Low emergence with infected seeds was also reported by Hartman *et al*. (1999). In current study it was observed that POE mortality was more when seeds with more than 50 per cent stained area were used.

Effect of purple stain on biochemical traits

Reduction in stearic acid, palmitic acid, linoleic and linolenic acid and increase in oleic acid, O/L ratio as well as oil content was observed with purple seed infection but the reduction and increase in category 2 to 6 was not consistent. However, the decrease in amount of TI and protein was consistent with the increase in seed infection category but the decrease in TI content was of high magnitude when stain was in 50 or more than 50 per cent surface area of seeds (Table 6). However, Prasanth and Patil (2007) recorded reduction in both protein and oil.

Table 6. Influence of purple seed stain on fatty acids, TI, protein and oil in NRC 7

Seed infection category	Fatty acid (%)					TI (mg/g)	Protein (%)	Oil (%)
	C16:0	C18:0	C18:1	C18:2	C18:3			
1	10.5c	2.76d	27.1a	53.1d	6.31b	75.5d	39.4 c	21.4a
2	9.6a	2.12a	34.4b	46.6a	5.89b	75.9d	38.8c	22.6b
3	9.9b	2.36c	34.7b	47.6c	5.36ab	74.4d	37.5bc	22.4b
4	9.5a	2.29bc	35.0b	47.5c	5.23ab	52.2b	36.9b	23.2c
5	9.5a	2.16a	40.0c	43.4a	4.76a	59.9c	36.2b	22.8b
6	9.6a	2.33b	35.7b	47.1c	5.18a	48.0a	34.4a	21.6a
C D (P = 0.05)	**0.210**	**0.146**	**2.340**	**3.191**	**0.750**	**2.61**	**1.360**	**0.41**

Values given are mean of triplicate samples on dry weight basis. Values with different superscripts in the same column are significantly (P<0.05) different

Table 7. Influence of purple seed stain on lipoxygenase activity and phenol content in NRC 7

Seed Infection category	Lipoxygenase (units/g of defatted soy flour)		Total Phenol (gallic acid equivalent/g)
	Lox I	Lox II+III	
1	1930c	592c	1.26b
2	1824a	570b	1.24b
3	1960d	608c	0.96a
4	1952c	669d	0.81a
5	1877b	610c	1.21b
6	1844a	544a	1.01a
C D (P = 0.05)	**26.40**	**18.11**	**0.156**

Values given are mean of triplicate samples on dry weight basis. Values with different superscripts in the same column are significantly (P<0.05) different

The trend in lipoxygenases and total phenol was inconsistent (Table 7). Xue *et al*. (2008) also reported that colonization of *C. kikuchii* was positively correlated with O/L ratio and oleic acid and negatively with linoleic and linolenic acid contents. Degeneration in seed coat protein and lipoxygenases were also reported by Velicheti and Sinclair (1992). Hartman *et al*. (1999) reported that seeds with 100 per cent discoloration tend to have lower oil content and higher protein content. Reduction in saturated fatty acids in the present study was in contrast with Xue *et al*. (2008) who observed no correlation with palmitic and stearic acid content and *C. kikuchii* colonization.

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Study of Changes in Some Parameters of Soybean in Response to Industrial Air Pollutants

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ABSTRACT

Atmospheric air is an aero-disperse system. In this gas-mixture, solid and liquid particulates are present. Pure air contains nitrogen, oxygen, noble gases and carbon-dioxide, but this ideal composition does not exist in nature since it is continuously getting polluted by pollutants emerging from industries. Soybean is widely used as components of food in all areas of the world. It has ability to grow under varying conditions of soil and climate. The present study was carried out with the aim to access the effect of industrial air pollutants on few physiological and biochemical parameters of soybean. Variations in the different parameters have been observed in response to industrial air pollution. Significant reduction in leaf extract pH (P-value = 0.009), total photosynthetic pigment (P-value = 0.009), ascorbic acid (P-value = 0.002), protein content (P-value = 0.0001) was observed at polluted site as compared to control site. However, reduction in relative water content (P-value = 0.19) and carbohydrate content (P-value = 0.078) was found insignificant at polluted site in comparison to control site. Change in air pollution tolerance index (APTI) was also calculated significant (P-value = 0.01) at polluted site, on the basis of above studied parameter. Thus, it was concluded that significant changes occurred in maximum parameters, except carbohydrates and relative water content in response to industrial air pollution. Hence, soybean crop is sensitive for pollution load in air.

Key words: Air pollution tolerance index, ascorbic acid, carbohydrate, industrial air pollution, protein, relative water content, soybean

Air pollutants like SO_2, NOx, SPM, and RSPM are responsible for reduction of biological and physiological responses of various plants and crops grown at polluted area (Chauhan and Joshi, 2008). At higher concentration of pollutants, plants are

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reported to exhibit visible injury symptoms and at lower concentrations, plants are reported to exhibit certain physiological and biochemical invisible injuries. Leaves are the most susceptible parts of a plant to acute injury due to abundance of stomata, which permit penetration of the air pollutants into the sensitive tissues. The first barrier of gaseous air pollutant is boundary layer resistance which varies with wind speed and size, shape and orientation of leaves (Heath *et al.,* 2009). The effects of SO_2 on physiological and biochemical characteristics of plants have been well documented (Agrawal *et al.,* 2006 and Chauhan and Joshi, 2010). The adverse effect of air pollution have been studied by taking some selected parameters separately such as relative moisture content (Joshi and Chauhan, 2008), chlorophyll content (Chauhan, 2008) and ascorbic acid content (Hoque *et al.,* 2007). Plants sensitivity and tolerance to air pollutants depends on parameters like leaf extract pH, relative water contents (RWC), ascorbic acid (AA) content, total chlorophyll content, *etc*. Study of single parameter may not provide a clear picture of the pollution induced changes; so air pollution tolerance index (APTI) which was based on these four parameters has been used to know tolerance levels of plant species (Liu and Ding, 2008; Singh and Verma, 2007) . APTI has been used to identify tolerance levels of plant species (Yan and Hui, 2008; Agbaire and Esiefarienrhe, 2009) and to select plant species tolerant to air pollution by landscapers (Yan and Hui, 2008). The soybean has been extensively used as important source of dietary protein and oil throughout the world. The crop plants are very sensitive to gaseous and particulate pollution and can be used as indicators of air pollution (Joshi and Swami, 2009). In a field transect study conducted by Chauhan and Joshi (2010), it was observed that area under higher load of pollutants (SO_2 6.5 ppb and NO_2 9 ppb) showed maximum reductions in growth and yield, ascorbic acid content and photosynthetic pigments of wheat and mustard crops. The present study was carried out with the aim to access the effect of industrial air pollutants on few parameters like relative moisture content, leaf extract pH, total photosynthetic pigments, ascorbic acid, APTI, protein and carbohydrate content of soybean crop.

MATERIAL AND METHODS

Experimental Area

Pithampur industrial area was selected for the present study. This industrial area was divided into three sectors 1, 2 and 3. Pattharmundla Gaon (agricultural area) was selected as non-polluted control area. List of industries situated at pithampur industrial area with their manufacturing products are given in Table 1.

Monitoring of air pollutants

The concentration of different air pollutants, *viz*. NOx, SO_2 was collected using respirable dust sampler and

Table 1. Details of Industries located in experimental area

Name of industry	Product manufactured	Name of industry	Product manufactured
Bhagiruth Couch	Eicher body	Mahle Magma	Cam shaft
Force Motors	Mini car, Jeep	Man Saw Pipe	Iron pipe
Neo Corp International	Packaging unit	Mahindra 2 Wheelers	Two wheelers assembly
Crompton Greaves	Railway signaling unit	Girnar Fileer	Cotton yarn
Rosy Blue	Diamond cutting	Indore Composite	FRP cable
VE Commercial Vehicle	Eicher bus	Caparo Unit – I	Sheet metal component
Caparo Unit- II	Long member	Ipca	Pharmaceutical products
Shiva Detergent	Detergent	Kirti Industrial	Plastic pipe
IPF Vikram	Detergent	Bridge Stone	Tyre
Nicholus Pharmaceutical	Pharmaceutical products	Cipla	Pharmaceutical/ Basic drugs
Symbiotic	Pharmaceutical/ Basic drugs	Prakash Solvex	Soybeans extraction plant/Refinery
Rit Spin	Cotton yarn	Mission	Pharmaceutical products
Dabur India	Hair oil, Tooth-paste	Pratibha Syntex	Cotton yarn and Fabrics
Spentex	Spindle, Cotton yarn	MID Indian	Steel work
Desingh Auto	Head light, Bulb production	Larsen and Turbo	Equipment/ Engineering unit
Tata Wire	Wire	Lupin	Pharmaceutical products

Fig. 1. Site plan of Pithampur industrial area

respirable suspended particulate matter (RSPM) monitored in the ambient air with the help of high volume sampler (HVS) by sucking air into appropriate reagent for 24 h at the selected area. The apparatus was kept at a height of 2 m from the surface of the ground. Once the sampling was over, the samples were brought to the laboratory and concentration of different pollutants was determined.

Determination of RSPM: Concentration of RSPM was determined using glass fiber filter paper methods. Air is drawn through a size-selective inlet and through a 20.3 cm x 25.4 cm filter at a flow rate of 1,132 L/min. Particles with aerodynamic diameter less than the cut-point of the inlet are collected, by the filter. The mass of these particles is determined by the difference in filter weights prior to and after sampling. The concentration of particulate matter (PM_{10}) in the designated size range is calculated by dividing the weight gain of the filter by the volume of air sampled.

Determination of NOx: Ambient nitrogen dioxide (NO_2) is collected by bubbling air through a solution of sodium hydroxide and sodium arsenite. The concentration of nitrite ion (NO_2^-) produced during sampling is determined calorimetrically by reacting the nitrite ion with phosphoric acid, sulfanilamide, and N-(1-naphthyl)-ethylenediamine di-hydrochloride and measuring the absorbance of the highly colored azo-dye at 540 nm (Jacob and Hochheiser, 1958).

Determination of SO_2: Sulphur dioxide from air is absorbed in a solution of potassium tetraChloro–mercurate (TCM). A dichlorosulphitomercurate complex, which resists oxidation by the oxygen in the air, is formed. Once formed, this complex is stable to strong oxidants such as ozone and oxides of nitrogen and therefore the absorber solution may be stored for some time prior to analysis. The complex is made to react with para-rosaniline and formaldehyde to form the intensely coloured pararosaniline methylsulphonic acid. The absorbance of the solution is measured spectrophotometerically at 560 nm (West and Geake, 1956).

Crop sampling sites

The present study was conducted by selecting soybean (JS 335) crop growing under field conditions in the Pithampur industrial area sector 1, 2 and 3 and in Pattharmundla Gaon as a control site. Samples of leaves and seed of soybean were collected. The fresh weight of leaves of all the samples was taken immediately upon getting to the laboratory. Samples were preserved in refrigerator for further analysis.

Biochemical analysis

All the Experiments were performed in triplicate and mean values were considered for analysis.

Photosynthetic pigment: The leaves were washed with distilled water and cut into small pieces. 100 mg each fresh leaves were taken and grounded with 5 ml of 80 per cent acetone in a mortar- pestle with a pinch of washed sand. The grounded samples were centrifuged, supernatant was collected and sediment was washed with 2 ml of 80 per cent acetone and again centrifuged for 3 minutes. The final volume of supernatant was made up to 10 ml by adding 80 per cent acetone. Absorbance was read in 645 to 663 nm for chlorophyll (a) and (b) respectively and 480 and 510 nm for carotenoids. The concentration of chlorophyll (a), (b) and carotenoid was calculated with the help of absorption coefficient of Arnon (1949). Following formulae were used:

Photosynthetic pigment (mg/g) of leaves = Chl.a + Chl.b+ Carotenoid

Chl.a (mg/g) = $22.7\times$ OD $663-2.69\times$OD 645,

Chl.b (mg/g) =$12.9\times$ OD $645-4.68\times$ OD 663

Carotenoied (mg/g) =$7.6\times$ OD 480 – $1.49\times$ OD 510

Relative water content (RWC): RWC = [FW-DW)/ (TW-DW)] x 100, where FW = fresh weight, DW= dry weight and TW= turgid weight.

Fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed in water over night, blotted dry and weighed to get turgid weight. Now the leaves were dried in an oven at 70 ˚C and reweighed to obtain the dry weight (Singh, 1977).

***Leaf extract pH**:* 5 g of the leaves were homogenized in 50 ml deionized water, and then filtered and the pH of filtered leaf extract was determined by using pH meter.

***Ascorbic acid content (AA)*:** Ascorbic acid content was determined by taking 1 g of the fresh foliage in a test-tube and 4 ml oxalic acid - EDTA extracting solution was added. Then 1 ml of orthophosphoric acid and 1 ml 5 per cent tetraoxosulphate (VI) was added to this mixture. Later 2 ml of ammonium molybdate was added followed by then 3 ml of water. The solution was then allowed to stand for 15 minutes. The absorbance was measured at

760 nm with a spectrophotometer (Bajaj and Kaur, 1981).

APTI was calculated by using following formula: APTI = [AA (T + P) + R] / 10, where R = Relative water content in mg/g, AA = Ascorbic acid in mg/g, T = Total chlorophyll in mg/g, and P = pH of leaf extract, (Singh and Rao, 1983).

Protein content: Protein content in the seed was estimated by taking the 100 mg sample, crushed with 10 ml NaOH and centrifuged at 3,000 rpm for 10 min, 0.2 ml supernatant was collected in centrifuge tube and diluted with 2 ml of distilled water and 3 ml of 10 per cent trichloro acetic acid (TCA) were added. The intensity of blue color was measured at 750 nm (Lowry *et al.,* 1951).

Total sugars: 200 mg of dried samples was hydrolyzed with 5 ml of 1 N H_2SO_4 at 50 ˚C for 30 minute. Then it was boiled for 80 minutes and one ml of aresnomolybdate solution was added. Deep blue colour appeared slowly. The volume was made up to 25 ml with distilled water and absorbance was read at 500 nm against blank by spectrophotometer (Malhotra and Sarkar, 1979)

RESULTS AND DISCUSSION

Air monitoring conducted in Pithampur industrial area has shown that sector -3 had maximum level of RSPM and NO_2 pollutants and concentration of SO_2 was found higher in Industrial Area Sec-1. Whereas the standard limit prescribed by Central Pollution Control Board (CPCB) of India for RSPM is 60-100 µg/m^3 for industrial, residential and sensitive areas, respectively, sulhpur dioxide (SO_2) 50-80 µg/m^3 and nitrogen dioxide 40-80 µg/m^3 (Table 1).

Variations in the different parameters (Table 2) revealed significant reduction in leaf extract pH (P-value = 0.009) at polluted area compared to control area. Reduction in relative water content was insignificant (P-value =0.19) at polluted site as compared to control site. Reduction in total photosynthetic pigment was extremely significant(P-value = 0.009) at polluted site as compared to control site. Reduction in ascorbic acid was extremely significant (P-value = 0.002) at polluted site as compared to control site. Change in APTI was significant (P-value = 0.01) at polluted site as compared to control site. Reduction in protein content was extremely significant (P-value= 0.0001) at polluted site as compared to control site Reduction in carbohydrate content was insignificant (P-value = 0.078) at polluted site. as compared to control site.

Leaf extract pH

Leaf extract also shown significant reduction in leaves of pollution affected site of soybean plants. SO_2 absorption within the cellular spaces produces reactive oxygen species (ROS) during chemical reaction (Bartoz, 1997), SO_2 readily dissolves in the apoplastic water to produce mainly sulphite (SO_3^{2}), bisulphate (HSO_3^-) and H^+ ions, reducing the PH of the medium (Legge and Krupa, 2002). SO_2, SO_3^{2-} and HSO_3^- ions are phototoxic (Dekok, 1990). Above studies also supports the reduction in pH due to presence of SO_2

Table 2. Twenty four hour mean pollutant concentration (μg/m³) during 2011 at different sites of Pithampur industrial area sector-1, 2 and 3

Sampling point	RSPM (μg/m³)	SO_2 (μg/m³)	NO_2 (μg/m³)
Industrial Area Sec.1,Sampling point: Near Force Motor, Pithampur	207.95	12.82	22.15
Industrial Area Sec.2, Sampling point: Near Piramal Health, Pithampur	113.28	9.59	19.06
Industrial Area Sec.3, Sampling point: Near Case New Holland, Pithampur	216.72	11.78	25.97
The standard limit prescribed by Central Pollution Control Board (CPCB) of India	60-100	50-80	40-80

alone in higher amount in atmosphere similarly NO_2, after entering in sub-stomatal cavity produces HNO_2, HNO_3 and H^+ ions (Ramage *et al.*, 1993), or forming free radical chain reaction (Sparks *et al.*, 2001), HNO_2 is also capable of initiating peroxidation process within lipid membrane (Ramage *et al.*, 1993).

Relative water content

The relative water content (RWC) defines the moisture holding capacity of a plant and is indicative of the hydration conditions in leaf matrix. RWC helps a plant to maintain its physiological balance under stress conditions like exposure to air pollution. Higher the RWC in a particular species greater is its drought tolerance capacity. Relative water content is associated with protoplasmic permeability in cells and causes loss of water and dissolved nutrients, resulting in early senescence of leaves (Agarwal and Tiwari, 1997). If leaf transpiration rate is reduced due to air pollution, plants lose ability to pull water and minerals from roots for biosynthesis. Higher relative water content in control than polluted site was observed. The present studies shown insignificant reduction in RWC of polluted area grown leaves of soybean crop. This little reduction of RWC also says something that air pollutants somehow damage protoplasmic permeability in cells and affect rate of transpiration too and thus soyabean crop's sensitivity is to be a point of measurement towards industrial air pollution.

Ascorbic acid

Ascorbic acid was reduced in leaves of soybean growing in industrially polluted area perhaps due to adverse effect of pollutants. Being an important reducing agent, ascorbic acid plays a vital role in cell wall synthesis, defense and cell division

Table 3. Values studied parameters of soybean crop (control *vs.* industrially polluted area)

Parameters	Control (Agricultural area)	Pithampur (Industrial area)			Mean value of parameter of sector 1, 2 and 3
		Sector-1	Sector-2	Sector-3	
Leaf extract pH	6.28 ± 0.01	6.24 ± 0.01	6.20 ± 0.01	6.17 ± 0.01	6.20 ± 0.03 (P <0.009)**
Relative water content	65.16 ± 2.50	65.25 ± 1.02 (+ 0.13 %)	65.08 ± 1.56 (0.12 %)	64.27 ± 1.03 (1.38 %)	64.87 ± 0.52 (P <0.19)^{NS}
Ascorbic acid (mg/g)	1.47 ± 0.02	1.40 ± 0.01 (4.76 %)	1.34 ± 0.01 (8.84 %)	1.38 ± 0.01 (6.12 %)	1.37 ± 0.03 (P <.002)**
Total Photo-synthetic pigment (mg/g)	2.74 ± 0.04	2.68 ± 0.02 (2.18 %)	2.62±0.02 (4.37%)	2.58±0.02 (5.83 %)	2.63 ± 0.05 (P <0.009)**
APTI	7.84	7.77	7.68 (2.04%)	7.63	7.69 ± 0.07
Protein content (mg/g)	350 ± 10	316.66 ± 15.27 (9.52 %)	320±10 (8.57 %)	310 ± 10 (11.42 %)	315.56 ± 5.09 (P < 0.0001)**
Carbohydrate content (mg/g)	314.28 ± 14.28	299.9 ± 14.28	314.23 ± 14.25	285.71 ± 14.29 (9.09	299.95 ± 14.26 (P < 0.078)^{NS}

*Note: P-value at 5% level, *, **, ^{NS} indicates very significant, extremely significant and non- significant, respectively*

(Conklin, 2001). It plays an important role in photosynthetic carbon fixation with the reducing power directly proportional to its concentration (Pasqualini *et al.*, 2001). Ascorbic acid, a stress reducing factor, is a strong anti-oxidant and is generally higher in tolerant plant species. The decrease in relative ascorbic acid content under polluted conditions indicated its sensitivity towards industrial air pollutants. Reduction in AA was found extremely significant that shows soybean crop's sensitivity towards air pollutants.

Total photosynthetic pigment

Effect of air pollution on chlorophyll deterioration is another factor of study to judge crop sensitivity against pollution load. Reduction in total photosynthetic pigment was found extremely significant in leaves of soyabean crop grown in polluted site that shows adverse effects of pollutants present in air of pithampur industrial area. Reductions in chlorophyll content of a variety of crop plants due to NO_2, SO_2 and O_3 exposure have also been reported by Agrawal and Deepak (2003). The reason for degradation of chlorophyll pigments can also be attributed to action of SO_2 and NO_2 on the metabolism of chlorophyll (Lauenorth and Dodd, 1981). The reduction in the concentration of chlorophyll might be caused due to the increase in chlorophyllase enzyme activities, which in turn affects the chlorophyll concentration in plants (Mandal and Mukherji, 2000). The chlorophyll was significantly low in the plants fumigated with different levels of SO_2 and chlorophyll a being more severely affected than chlorophyll b. Chlorophyll a is degraded to phaeophytin through replacement of Mg^{+2} ions in chlorophyll molecules, while chlorophyll b forms chlorophyllide b through the removal of phytol group of the molecule (Wali *et al.*, 2004). Extremely significant reduction in total chlorophyll content in soybean leaves of polluted sites shows adverse effects of pollutants present in Pithampur industrial area. Which is might be possible because of any biochemical change in chlorophyll synthesis process, as mention in above studies.

APTI content

Reduction in APTI was less than 10 per cent in industrial area which indicates sensitivity of soybean (variety JS 335) towards industrial air pollutants. Thus, SO_2, NO_2 and other pollutants causes visible and invisible injury with ultra structural changes has been linked to reduction in transportation and photosynthesis and subsequently makes the plants difficult in growth and survival. Reduction in air pollution tolerance index (APTI) is a good parameter to know the tolerance capacity of plants against pollution. APTI was also reduced in the present study, which is a strong indicator that Soybean variety JS 335 is sensitive to air pollutants.

Protein content

Reduction in protein content was due to the enhanced rate of protein denaturation which is also supported by the findings of Prasad and Inamdar (1990). Constatinidou and Kozlowski (1979) found that enhanced protein denaturation and breakdown of existing protein to amino acid was the main causes of reduction in protein content. Denaturation and breakdown of protein is another reason of showing down the metabolic process in plants growing under stress of air pollution. Extremely significant reduction in protein content was observed in study area.

Sugar content

Pollutants like SO_2, NOx and H_2S under hardening condition can cause more depletion of soluble sugars in leaves of plants grown in polluted area (Davison and Barnes, 1986). Reduction in soluble sugar content in industrial area can be attributed to increased respiration and decreased CO_2 fixation because of chlorophyll deteriorations.

The studies of air pollutants in Pithampur industrial area shown that RSPM crossed the permissible limits as suggested by CPCB, however other pollutants like SO_2, NO_2, were under the limits. But even then they have adverse effects on physiology and biochemistry of plant life. Present work showed that changes in biochemical parameters in response to air pollutants. The study clearly shows that gaseous (NO_x and SO_2) and particulate pollutants such as RSPM have detrimental effects on Soybean crops. Changes in photosynthetic pigment and biochemical parameters of Soybean crops directly corresponded to the levels of air pollution at different sectors of Pithampur industrial area. The study elucidates that air pollution emitted from industries adversely affecting the ambient air and agricultural production. It is very clear that industrial air pollution has become a serious threat to agricultural production grown adjacent to industrial areas. More research is, however, necessary to evaluate the contribution of individual and in combination of air pollutant on crop production. From the above study it was concluded that physiological and biochemical parameters of soybean crop were adversely affected by industrial air pollution. All the studied biochemical parameters of soybean crop are vulnerable to air pollutants in various extents and shown reduction in amount lesser or greater. APTI, Index of tolerance was also found reduced due to pollution load. Thus, the soybean variety JS 335 was altogether found sensitive to air pollution and cannot be suggested to grow in and nearby industrial area like pithampur where various factories imitating their pollutants in atmosphere and contributing pollution load. Hence, farmers should not grow this soybean variety preferably around industrial sectors.

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Economics of Soybean Cultivation and Analysis of Production Constraints in Central Narmada Valley of Madhya Pradesh

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ABSTRACT

The present study was undertaken to work out the cost of cultivation, profitability and constraints of soybean production at different size of farms in central Narmada valley of Madhya Pradesh. The study is based on primary data collected from 100 cultivators belonging to three categories namely, small farms (40), medium farms (35) and large farms (25). The cost of soybean cultivation in case of medium farms was found to be higher (INR 26,253/ha) in comparison to small farms (Rs 24,623/ha) and large farms (Rs 24,536/ha). The Cost A_1 of soybean cultivation accounted for 54.92 per cent, 56.61 per cent and 58.56 per cent on small, medium and large farms, respectively of the total cost of cultivation. The percentage of cost A_1 in cultivation of soybean was found to increase with the increase of size of farms. The maximum gross income from soybean cultivation observed on medium farms was INR 41,130 followed by small (Rs. 38989/ha) and large (Rs. 37902/ha) farms. The highest benefit cost ratio was reported from small farms (1:1.58) followed by medium (1:1.57) and large (1.1.54) farms. The soybean cultivators reported that potential yield could not be achieved due to lack of hired human labour during the cropping season, soil testing facilities to optimize nutrient management, knowledge on recommended package of practices including crop protection practices and intercropping techniques, and capital for investment.

Key words: B: C ratio, cost of cultivation, gross income, profitability, soybean

Soybean [*Glycine max* (L.) Merrill] belongs to the legume family, is recognized as golden or miracle bean due to its high nutritive value and allied uses. Soybean seed is rich in protein (40 %) and edible oil (20 %). Among the oilseeds, soybean ranks first in the world edible oil production (57 %) and international trade. It occupies a premier position among nine major oilseeds in India from last few years and during 2011 it is estimated that the crop covered an area of 10.18 million hectares producing 12.28 million tonnes with productivity of 1,208 kg per ha. In the leading state, Madhya Pradesh, the crop was cultivated in 5.67 million hectares producing 6.28 million tonnes with productivity of 1108 kg per ha (-http://eands.dacnet.nic.in/ Publication 12- 12- 2012 / Agriculture_ at _a_ Glance % 202012 / Pages 85 - 136. pdf).

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The crop also plays an important role in the national economy by way of contributing about 25 per cent in edible oil production and earning about INR 7000 crores through export of soya meal. The prospective in terms of income and export potential and ensures the highest profit making among major *kharif* crops of the State (Gautam and Nahatkar, 1993). The major problem in soybean production is the stagnant productivity in the country in general and especially Madhya Pradesh in particular (Nahatkar *et al.*, 2005), especially when varieties with high yield potentials and improved production technology is available. The present study aims at working out the cost of soybean cultivation on different sized farms with estimation of profitability and possible constraints restricting the productivity.

MATERIAL AND METHODS

The present study was confined to Central Narmada valley agro-climatic region of Madhya Pradesh, which covers three districts namely, Narsinghpur, Harda and Hoshangabad. On the basis of homogeneity with respect of agro-climatic development, the Harda district was randomly selected for this study. Three stage random sampling design, *i.e.* block, village and soybean growers, was followed for sample selection for the study. Harda block of the district was selected randomly and two villages of this block were further randomly selected for collection of primary data from soybean cultivators. A list of all the soybean growers of these two selected villages was prepared and categorized into small (< 2 ha), medium (2 to 4 ha) and large (> 4 ha) based on the operational holding (Prasad, 1975). Further 10 per cent of soybean growers were selected from each category. Thus, the sampling unit comprised of 100 soybean cultivators (40 small, 35 medium and 25 large). The data were collected through pre-tested interview schedule from sample cultivators for the agricultural year 2012-13.

The different cost components, namely hired human labour, family labour, machine power, seed, plant protection materials, fertilizer, interest on working capital, land revenue, rental value of owned land, depreciation and interest on fixed capital were taken into consideration for the study. The cost of cultivation of soybean was estimated under various cost concepts *viz.*, Cost A_1, Cost A_2, Cost B_1, Cost B_2, Cost C_1, Cost C_2 and Cost C_3. The cost concepts were used for estimation of soybean cultivation, which are adopted by Commission for Agricultural Cost and Price, GOI. The gross income, net income, and benefit cost ratio were also worked out using different profitability concepts. The collected data were processed to work out the profitability and identify the constraints restricting the enhancement in soybean yield.

RESULTS AND DISCUSSION

Cost of cultivation of soybean

The investment made in inputs for cultivation of soybean by the selected farmers was analysed (Table 1). The results indicated that, soybean growers of Harda district of Madhya Pradesh invested on an average INR 25,186 per ha in cultivation of soybean. The cost of cultivation of soybean was found to be maximum on medium (Rs 26,253/ha) followed by small (Rs 24,623/ha) and large (Rs 24,536/ha) sized farms. The farmers of medium farm size group incurred higher cost on plant protection chemicals, machine labour and hired human labour. Similar findings were also reported by Rajput (2001). As regard to Cost A_1, the maximum cost A_1 on soybean cultivation was incurred by medium (Rs 14,861/ha) farmers followed by large (Rs 14,368/ha) and small (Rs 13,522/ha) farmers, which constituted 56.61 per cent, 58.56 per cent and 54.92 per cent, respectively, of the total cost of cultivation. As regards to different components of cost, the large farmers invested more on seed (17.95 %) as compared to small farmers (16.07 %) and medium farmers (15.67 %) of the total cost of soybean cultivation. Sharma *et al*. (1992) reported that rental value of land accounted for the highest share of total cost followed by seed, hired human labour, bullock labour and imputed family labour. It is also observed (Table 1) that large farmers invested more on use of machines (10.68%) than human labour followed by medium (10.35 %) and small (10.34 %) farmers. High cost labour and available mechanisation on large farms might be the reason for shift from human labour to use of machines for farm operations.

A significant proportion of total cost of cultivation was incurred on plant protection chemicals in the study area, may be mainly due to the monoculture over the years resulting in susceptibility of crop for crop pests and diseases.

In total cost of cultivation of soybean, the rental value of land (25.79 %) was found to be main component of cost followed by seed (16.46 %), use of machine power (10.44 %), plant protection material (8.67 %), fertilizer (8.42 %), family labour (5.30 %) and hired human labour (4.73 %). Similar finding were also observed by Idnani *et al.* (1992) and Sharma *et al.* (1996).

Profitability from soybean cultivation

The profitability of soybean cultivation in Harda district was analysed and presented on Table 2. It is evident from the results, that average yield of soybean in the study area was 1,280 kg/ha. The soybean yield was highest on medium farms (1,343 kg/ha) followed by small (1,286 kg/ha) and large (1,200 kg/ha) farms. This might be due to the fact that medium farmers used their scare resources more efficiently as compared to small and large farmers. The large farmers sold their product at higher rate (Rs 30.73/kg) than medium farmers (Rs 30.10/kg) and small farmers (Rs 29.55/kg) as they had better technical knowhow and produce retaining (storage) capacity facilitating disposal of produce on higher market price.

The economic evaluation (Table 2) revealed that an average farmer received Rs 39,440 per ha and Rs 14,254 per ha respectively, as gross and net returns with 1:1.56 as B:C ratio the cultivation of soybean. The medium farmers received higher returns (gross Rs 41,130/ha and net – Rs 14,877/ha) as compared to small

Table 1. Cost of cultivation (Rs/ha) of soybean crop at different farms sizes

Cost concept	Cost items	Size of farm			
		Small	**Medium**	**Large**	**Average**
Cost A_1	Hired Human labour	1219 (4.95)	1276 (4.86)	1058 (4.31)	1191 (4.73)
	Machine labour	2546 (10.34)	2717 (10.35)	2621 (10.68)	2628 (10.44)
	Seed	3957 (16.07)	4113 (15.67)	4405 (17.95)	4146 (16.46)
	Fertilizer	2342 (9.51)	2247 (8.56)	1714 (6.99)	2120 (8.42)
	Plant Protection Chemicals	1842 (7.48)	2456 (9.35)	2260 (9.21)	2182 (8.67)
	Depreciation	550 (2.24)	751 (2.86)	931 (3.79)	735 (2.92)
	Land Revenue	18 (0.07)	20 (0.08)	20 (0.08)	19 (0.08)
	Interest on working capital	1048 (4.26)	1281(4.88)	1358 (5.53)	1238 (4.92)
Cost A_2	Cost A_1	13522 (54.92)	14861 (56.61)	14368 (58.56)	14261 (56.62)
	Rent paid for leased in land	0	0	0	0
	Cost A_2	**13522** (54.92)	**14861** (56.61)	**14368** (58.56)	**14261** (56.62)
Cost B_1	Cost A_1	13522	14861	14368	14261
	Interest on Fixed capital	648 (2.63)	838 (3.19)	947 (3.86)	804 (3.19)
	Cost B_1	**14170**	**15699**	**15315**	**15065**
Cost B_2	Cost B_1	14170	15699	15315	15065
	Rental value owned value	6464 (26.25)	6835 (26.04)	6134 (25.00)	6495 (25.79)
	Cost B_2	**20634**	**22534**	**21448**	**21560**
Cost C_1	Cost B_1	14170	15699	15314	15065
	Imputed value of family labour	1750	1332	857	1336
	Cost C_1	**15920**	**17031**	**16171**	**16401**
Cost C_2	Cost B_2	20634	22534	21448	21560
	Imputed value of family labour	1750 (7.11)	1332 (5.07)	857 (3.49)	1336 (5.30)
	Cost C_2	**22384**	**23866**	**22305**	**22896**
Cost C_3	Cost C_2	22384	23866	22305	22896
	Cost C_2 of 10 %	2238 (9.09)	2387 (9.09)	2230 (9.09)	2290 (9.09)
	Cost C_3	**24623**	**26253**	**24536**	**25186**
		100	**100**	**100**	**100**

Figures in parentheses indicate percentage farmers (Rs 38,989/ha and Rs 14,366/ha, respectively) and large farmers (Rs 37,902/ha and Rs 13,366/ha, respectively). Although, the presented results do not precisely corroborates the findings of Rajput (2001) stating that net returns from soybean cultivation was highest on large size of land holding followed by medium and small size of land holding, but brought out that soybean cultivation on small land holdings produced less and led to lower economic advantage. However, the C:B ratio revealed a inverse relationship with size of land holding. This suggested that the small farmers (B: C:: 1:1.58) used their scare resources optimally as compared to medium (B:C::1:1.57) and large farms (B:C::1:1.54) in soybean cultivation, and in turn achieved higher returns from per rupee invested on soybean cultivation.

Constraints of cultivation of soybean

The data (Table 3) revealed that lack of hired human labour during peak operational periods was the main constraint in the study area in cultivation of soybean as reported by 74 per cent of cultivators, missing soil testing facilities (72 %), high cost of inputs (70 %), lack of knowledge on plant protection measures (63 %), inadequate capital (54 %), timely non-availability of quality seed of improved variety (53 %), Missing awareness on intercropping techniques (51%) and deficit in knowledge on recommended production practices (49 %) were the other major constraints reported by the soybean growers in the study area. Most of the farmers of all farm size categories experienced these constraints, but frequency was higher on smaller farm holdings than large holdings.

Table 2. Profitability from soybean cultivation at different size of farms

Particulars	Size of farms			
	Small	**Medium**	**Large**	**Overall**
Main Product (kg/ha)	1286	1343	1200	1280
Price of main product (Rs/kg)	29.55	30.10	30.73	30.10
Value of main product (Rs/ha)	38001	40424	36876	38528
By product (kg/ha)	1300	1086	1283	1233
Price of by product (Rs/kg)	0.76	0.65	0.80	0.74
Value of by product (Rs/ha)	988	706	1026	912
Gross returns (Rs/ha)	38989	41130	37902	39440
Net returns (Rs/ha)	14366	14877	13366	14254
B:C ratio	1: 1.58	1: 1.57	1: 1.54	1:1.56

Earlier workers (Ahirwar *et al.*, 2006; Sharma *et al.*, 1996) also reported that the soybean growers were not able to harness the yield potentials on account of constraints like high cost of inputs, unavailability of desired inputs at right time, lack of knowledge on intercropping techniques, capital deficit and non- availability of proper equipments for cultivation, especially seed drills for planting intercrops (maize and pigeonpea) at recommended spatial arrangement with appropriate seed rate. However, intercrop seed drill is a reality now and is developed and validated at Directorate of Soybean Research, Indore.

Table 3. Constraints of soybean cultivation at different size of farms

Constraints	Size of farms			
	Small (40)	Medium (35)	Large (25)	Total (100)
Deficit in knowledge on recommended package of practices	23 (57)	15 (43)	11 (44)	49 (49)
Non-availability of quality seed of improved varieties in time for sowing	22 (55)	18 (51)	13 (52)	53 (53)
Non availability of fertilizers in time	25 (71)	15 (42)	10 (40)	50 (50)
Lack of hired human labour at peak operational periods	34 (85)	25 (71)	15 (60)	74 (74)
Missing soil testing facility	33 (82)	20 (57)	19 (54)	72 (72)
Inadequate capital	27 (67)	17 (48)	10 (40)	54 (54)
Lack of knowledge of plant protection methods	29 (72)	23 (65)	11 (44)	63 (63)
High cost of inputs	32 (80)	23 (66)	15 (60)	70 (70)
Missing awareness on intercropping techniques	24 (60)	17 (48)	10 (40)	51 (51)

Parenthesis shows percentage of total farms

The information analyzed and presented in this paper suggests that the soybean productivity and profitability of soybean cultivation at medium farms is comparatively higher than small and large farm holdings. However, the inverse relationship of B:C ratio with farm size revealed that the returns per rupee invested is higher on small farms as these farmers optimally used their scarce resources. The farmers perceived and identified constraints paves the way for all stakeholders in soybean production to make the efforts to address them towards enhancing the productivity of soybean.

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Impact of Integrated Input Resource Management on Production and Nutrient Uptake of Soybean [*Glycine max* (L.) Merrill]

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Soybean [*Glycine max* (L.) Merrill], a major legume crop, is recognized as the efficient producer of two scarce nutritional resources, namely, protein and oil, which are not only the major components in the diet of vegetarian masses but a boon to the developing countries for mitigating energy-protein malnutrition. Farm- yard manure (FYM) is the main source of primary, secondary and micronutrients to the plants. *Rhizobium* and phosphorus solublizing bacteria (PSB) are the important biofertilizers as component of integrated nutrient management. Micronutrients mainly zinc and molybdenum has key role in the crop development and sustainability. Integrated input resource manage-ment involves use of manures, biofertilizers and chemical fertilizers, all together, to achieve sustainable crop production. The present investigation was planned to study the impact of integrated input resource management on the productivity and nutrients uptake of soybean crop.

The experiment was conducted during *kharif* 2006 with soybean - wheat cropping sequence employing split plot design involving three treatments based on application of nutrients on soil test basis in main plots, namely optimum dose of NPKS, 75 per cent of the optimum dose of NPKS, 50 per cent of the optimum dose of NPKS through different sources. The soil test based nine sub- plot treatments included 100 per cent NPKS, 75 per cent NPKS + 5 t FYM per ha, 50 per cent NPKS+ 5 t FYM per ha, 75 per cent NPKS + 5 t FYM per ha + *Rhizobium japonicum* @ 10 gper kg seed, 75 per cent NPKS + 5 t FYM per ha + PSB @ 1.5 kg per ha, 75 per cent NPKS + 5 t FYM per ha + PSB @ 1.5 kg per ha + *Rhizobium japonicum* @ 10 g per kg seed, 75 per cent NPKS + 5 t FYM per ha + PSB @ 3 kg per ha +

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Rhizobium japonicum @ 10 g per kg seed + Zn @ 10 kg per ha, 75 per cent NPKS + 5t FYM per ha + PSB @ 3 kg per ha + *Rhizobium japonicum* @ 10 g per kg seed + Mo @ 500 g per ha and 75 per cent NPKS + 5 t FYM per ha + PSB @ 3 kg per ha + *Rhizobium japonicum* @ 10 g per kg seed + Zn @ 10 kg per ha + Mo @ 500 g per ha. The size of main plot and sub-plot was 18 m x 6 m and 6 m x 6 m, respectively.

Soybean variety JS 90-41 was raised following the recommended package of practices. The crop was sown on 2nd July, 2006 and harvested on 4th October, 2006.

The optimum dose of NPKS for soybean was 20 kg N, 80 kg P_2O_5 and 20 kg K_2O per ha, which was applied through urea, single superphosphate, and muriate of potash. Zn and Mo were supplied through zinc sulphate (21% Zn) and ammonium molybdate (52% Mo) in combination with FYM. Soybean seeds were inoculated with *Rhizobium japonicum* and PSB was incorporated in soil before sowing of the crop. The FYM @ 5 t per ha, applied as basal, analyzed 0.42 per cent N, 0.23 per cent P_2O_5, 0.74 per cent K_2O and 0.20 per cent S.

The soil of the experimental site analyzed: pH 6.94 (Piper, 1966), organic carbon 0.89 per cent (Walkley and Black, 1934), available nitrogen 313 kg per ha (Subbiah and Asija, 1956), available phosphorus 26.4 kg per ha (Olsen *et al.*, 1954), available potassium 342 kg per ha (Muhr *et al.* 1965), available sulphur 20.5 mg per kg (Chesnin and Yien, 1951) and available zinc 0.52 mg per kg (Lindsay and Norvell 1978).

Plant samples were collected at 45 days after sowing (DAS), 60 DAS and grain and straw yields were recorded at harvest of the crop. Plant samples were dried in an oven at 60^{0}C, for 72 hours and then dry weight was recorded. Plant biomass as well as the grain and straw from all treatments were analyzed for N (AOAC, 1965), P (Koenig and Johnson, 1942), K (Black, 1965), S (Bardsley and Lancaster, 1960) and Zn (Lindsay and Norvell 1978) contents.

Effect of treatments on growth and yield parameters

The data on biomass production at 45 DAS, 60 DAS and at harvest revealed significant differences due to 100, 75 and 50 per cent NPKS levels, respectively. The maximum biomass recorded was from 100 per cent NPKS and minimum at 50 per cent NPKS levels, at all the growth stages. Among sub-plot treatments, maximum biomass production was recorded from the treatments receiving 75 per cent NPKS + 5 t FYM + biofertilizers in conjun-ction Zn and/or Mo at all the stages of observations. These treatments were significantly superior to remaining treatments. The values for biomass production at harvest ranged between 4160 kg per ha (75 % NPKS/ha + 5 t FYM/ha + PSB + *Rhizobium* + Zn + Mo) and 3,263 kg per ha (50 % NPKS/ha + 5 t FYM/ha).

The yield data of seed and straw (Table 1) for main plots revealed a gradual decrease with decease in application of NPKS, maximum being with 100 per cent NPKS (1,935 kg/ha) and minimum with 50 per cent NPKS (1,273 kg/ha). The B:C ratio, irrespective of sub-plot treatments, was also higher with 100

per cent NPKS (1.82) as compared to 50 per cent NPKS (1.16). Among sub-plot treatments, the maximum seed (1692 kg/ha) and straw (2470 kg/ha) yields were associated with 75 per cent NPKS per ha + 5 t FYM per ha + PSB + *Rhizobium japonicum* + Zn + Mo, whereas minimum (1,343 and 1,920 kg/ha, respectively) with 50 per cent NPKS per ha + 5 t FYM per ha. Former treatment was on par with 75 per cent NPKS per ha + 5 t FYM per ha + PSB + *Rhizobium japonicum* + Zn or Mo. The B:C ratio of sub-plot treatments revealed invariably higher values when biofertilizers (1.55 - 1.71) and Zn and Mo (1.68 - 1.77) were integrated as compared to 100 per cent NPKS (1.53) indicating the beneficial effect of these components in increasing yield and profitability. The improvement in the crop growth and yield was due to better utilization of the applied resources involving integrated approach. Similar results had earlier been also reported by Mishra *et al.* (2005), Singh *et al.* (2008) and Thakur *et al.* (2011).

Effect of treatments on nutrients uptake

The data on uptake of N, P, K, S and Zn nutrients by soybean seed and straw clearly indicated that the uptake of N, P, K, S and Zn (Table 2) synchronized with the biomass produced and it was highest in 100 per cent NPKS treatment.

Increasing rates of fertilizer application (main plots) successively increased the uptake of these nutrients by the crop. The uptake of N, P, K and S in soybean seed, which was 60.80, 6.67, 37.97 and 1.15 kg per ha, respectively in 50 per cent NPKS, increased to 96.90, 11.24, 71.84 and 2.92 kg per ha due to the application of 100 per cent NPKS. Further, the uptake of Zn by soybean seed was found to be maximum (960.48 g/ha) in 100 per cent NPKS and minimum in 50 per cent NPKS (617.26 g/ha). Similar was the trend in straw also. In case of sub-plot treatments, reducing the level of fertilizers with introduction of FYM did not show improvement in nutrient uptake. With the introduction of micronutrients (Zn or/and Mo) and biofertilizer resource (Rhizobium or/and PSB) with 75 per cent NPKS and FYM, the uptake values of considered nutrients raised to either 100 per cent NPKS level or higher than that. Such a behavior was expected due to better biological activities in the presence of biofertilizers and the micronutrients. Similar results also reported by Kumar *et al.* (2012).

On the basis of the results of this experiment it can be inferred that integrated resource management practice based on soil test value could improve nutrients uptake and yield coupled with higher B:C ratio, particularly when the rate of nutrients application is 25 per cent below the recommended level.

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Table 1. Biomass production, seed and straw yield and B:C ratio of soybean

Treatments	Biomass production (kg/ha)			Yield (kg/ha)		B:C ratio
	45 DAS	60 DAS	At har-vest	Seed	Straw	
Main plot						
100 % NPKS	1608	2493	4798	1935	2863	1.82
75 % NPKS	1506	2271	3631	1541	2090	1.49
50 % NPKS	1395	2018	3168	1273	1895	1.16
S Em (±)	4.3	7.7	20.3	19.9	13.8	-
CD (P = 0.5)	**7.1**	**30.4**	**80.1**	**78.2**	**54.3**	
Sub- plot						
100 % NPKS	1390	2069	3672	1564	2108	1.53
75 % NPKS + 5 t FYM	1319	1969	3523	1456	2067	1.40
50 % NPKS + 5 t FYM	1224	1830	3263	1343	1920	1.27
75 % NPKS + 5 t FYM + *Rhizobium*	1450	2145	3831	1567	2264	1.59
75 % NPKS + 5 t FYM + PSB	1519	2233	3790	1540	2250	1.55
75 % NPKS+5t FYM +PSB +*Rhizobuum*	1588	2380	4027	1646	2380	1.71
75 % NPKS + 5 t FYM + PSB + *Rhizobium* + Zn	1663	2482	4132	1649	2483	1.68
75 % NPKS + 5 t FYM + PSB + *Rhizobium* +Mo	1713	2553	4098	1690	2405	1.77
75 % NPKS + 5t FYM + PSB + *Rhizobium* + Zn + Mo	1720	2560	4160	1692	2470	1.72
S Em (±)	7.7	10.8	29.7	18.9	20.6	-
CD (P =0.05)	**22.4**	**31.4**	**86.0**	**54.6**	**59.6**	

Table 2. Nutrient uptake of soybean at harvest

Treatments	Nutrient uptake									
	N (kg/ha)		P (kg/ha)		K (kg/ha)		S (kg/ha)		Zn (g/ha)	
	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw
Main plot										
100% NPKS	96.90	45.66	11.24	3.98	71.84	48.31	2.92	2.34	960.48	356.89
75 % NPKS	73.81	31.90	8.36	1.97	51.22	36.17	2.09	1.13	682.56	231.23
50 % NPKS	60.80	23.87	6.67	1.63	37.97	29.58	1.15	0.94	617.26	172.00
S.Em. (±)	0.929	0.181	0.122	0.012	0.586	0.668	0.024	0.072	16.986	2.276
CD (P = 0.05)	**3.647**	**0.714**	**0.480**	**0.049**	**2.304**	**2.624**	**0.094**	**0.286**	**66.684**	**8.936**
Sub-plot										
100 % NPKS	76.13	27.51	8.14	1.69	50.34	29.12	1.40	0.92	652.11	161.89
75 % NPKS + 5 t FYM	67.06	22.51	7.30	1.44	43.21	26.45	1.06	0.82	576.82	140.22
50 % NPKS + 5 t FYM	60.07	20.25	6.52	0.97	37.20	22.12	0.71	0.37	499.16	106.00
75 % NPKS + 5t FYM + *Rhizobium*	76.57	30.03	8.46	2.21	52.16	36.63	1.72	1.03	656.77	191.78
75 % NPKS + 5t FYM + PSB	75.71	33.86	8.60	2.22	53.35	37.96	1.96	1.26	673.00	203.45
75 % NPKS + 5t FYM + PSB + *Rhizobium*	81.18	38.03	9.37	2.85	58.05	40.84	2.11	1.40	735.22	288.67
75 % NPKS + 5 t FYM + PSB + *Rhizobium* + Zn	82.02	42.54	9.12	3.06	47.62	41.60	2.44	1.90	974.00	405.00
75 % NPKS + 5 t FYM + PSB + *Rhizobium* + Mo	85.02	41.33	10.11	3.43	61.91	45.09	3.04	2.09	880.11	322.22
75 % NPKS + 5 t FYM + PSB + *Rhizobium* + Zn +Mo	86.79	43.21	10.13	3.90	62.95	46.24	3.07	2.45	984.00	410.11
S.Em. (±)	0.876	0.610	0.014	0.024	0.606	1.254	0.025	0.124	10.870	6.396
CD (P = 0.05)	**2.527**	**1.760**	**0.392**	**0.069**	**1.751**	**3.619**	**0.087**	**0.358**	**31.360**	**18.453**

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Microbial Utilization of Soybean Processing Mill Wastes for Composting

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In recent past, India has surpassed China in terms of sown area under soybean. The spread of soybean in different parts of the country also resulted into parallel growth of oil industries by commissioning solvent extraction plants, which, apart from expelling oil, are also earning foreign exchange through export of deoiled cake (DOC). During processing of soybean in extraction plants, it is first subjected to cleaning, grading, sieving and de-hulling, generating trash/wastes materials like fodder sticks, broken grain pieces and dust particles. Till date, no economical use of these soybean wastes is in practice. During 2010-11 in India, total crushing of soybean was about 8.5 MT, which generated about 4,250 tons of soybean waste which is being dumped (SOPA 2011- personal communication). The microbial population of soil is comprised of five major groups *viz.*, bacteria, actinomycetes, fungi, algae and protozoa; bacteria being most abundant (Alexander, 1961) and important for decomposing wastes. Composting is the controlled biodegradation and transformation of organic materials, usually under aerobic conditions into an end product which is stable and called compost. An important source of thermostable enzymes can be found in the bacterial kingdom, where thermophilic prokaryotes are distributed among various groups in the phylogenic tree of bacterial and archaeal domains (Kristjansson and Stetter, 1992). The soybean processing mill waste is also called as "soybean lees" having higher nitrogen content (1.5 to 2 %), which could be used as a better substrate for rapid decomposition and with possibility of having degrading microbes capable to serve as starter inoculum for rapid compositing of wheat and rice straw. Therefore, keeping in view the importance of wastes as value addition, the present investigation was aimed to exploit soybean wastes from soybean mills

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around Indore for isolation of hydrolytic bacteria (proteolytic, cellulolytic, amylolytic and lipolytic).

Collection of wastes: About 5 kg of different types of soybean lees were collected for experimentation from two soya processing plants [Vippy Industries (food grade) and Premier Industries (animal feed grade)] located in Dewas (Table 1).

Selection of soya processing wastes for the study: Based on total N and organic carbon contents (Table 2), four types of wastes were selected for the study (Table 3). The samples were air dried and wet digested for estimation of total N using Kjeldahl method (Bremner, 1960). Organic carbon was determined by standard potassium dichromate and back titration with Fe^{++} solution (Walkley and Black, 1934).

Processing of wastes for decomposition and isolation of bacterial strains: Selected soybean lees mixed in different combinations with cow dung alone and with wheat straw and cow dung (Table 3) and were processed for decomposition using semi-solid-anaerobic system in buckets at moisture content of about 45-50 per cent. The containers covered with moist gunny bags at the top were kept at room temperature in the dark. Samples were drawn at 40, 60, 80 days for the isolation of hydrolytic bacteria. Aliquot of decomposing waste was subjected to serial dilutions and isolates were recovered on specific solid media using casein agar-proteolytic (Smith *et al.*, 1952) for proteolytic bacteria, starch agar-amylolytic (Gunasekaran, 1991) for amylolytic bacteria, CMC agar-cellulolytic (Gunasekeran, 1991) for cellulolytic bacteria and lipase agar-lipolytic (Gunasekeran, 1991) for lipolytic bacteria.

Table 1. Details of collected soybean waste materials

Sample details	Location
Hulls (V) Dust (V) Dust + hulls mix (V) Dust mixed with broken pieces of grains (V) De-oiled cake and burn waste (V) Straw (V)	Vippy Industries, Dewas (Food grade processing plant)
Dust+hulls mix (P) Dust (P)	Premier Industries Ltd, Dewas (Feed grade, processing plant)

Table 2. Total N (%) and organic carbon (%) of selected soybean wastes

Soybean waste	Total N (%)	Organic carbon (%)
Hulls (V)	1.58	39.9
Dust (V)	3.73	16.9
Dust + hulls mix (P)	2.38	25.4
Dust (P)	2.61	19.9

Isolation of hydrolytic bacteria: Proteolytic characteristic of bacterial isolates was observed by pouring solution of mercuric chloride (15 g $HgCl_2$ in 20 mL concentrated HCl made up to 100 mL with distilled water) on the culture plates (Smith *et al.*, 1952). Hydrolysis of casein was exhibited by a clear zone in a milky background which in turn is indicative of extracellular protease being secreted by the bacteria. Amylolytic property of bacteria was characterized by flooding the culture plates with iodine solution (0.3 % iodine

Table 3. Total N (%) and organic carbon (%) of soybean wastes amended with wheat straw and cow dung

Treatment	Total N (%)	Organic carbon (%)
Hulls (V) + wheat straw + cow dung (1:4:0.5)	1.21±0.15	19.33±0.12
Dust (V) + wheat straw + cow dung (1:4:0.5)	1.54±0.08	20.03±0.22
Dust +hulls (P) + wheat straw + cow dung (1:4:0.5)	1.16±0.15	20.37±0.15
Dust (P) + wheat straw + cow dung(1:4:0.5)	1.30±0.11	19.03±0.17
Hulls (V) + cow dung (10:1)	2.8±0.21	30.51±0.85
Dust (V) + cow dung (10:1)	1.63±0.30	9.0±0.15
[Dust + hulls (P)] + cow dung (10:1)	2.10±0.25	11.05±0.31
Dust (P) + cow dung (10:1)	1.93±0.20	6.98±0.07

V- Vippy Industry food grade; P- Premier Industry feed grade

and 1.0 % KI) and then observing the presence of clearing zone around the bacterial colonies in a blue background. The lipolytic property of bacteria was indicated by a clear zone while incubating at 37°C for 24-48 h in a turbid background. Cellulolytic activity of isolated bacteria was monitored by congored overlay method. In this method plates were flooded with 0.1 per cent aqueous congored solution for 10 minutes. Thereafter, they were washed with 1M NaCl solution. Colonies showing perceptible zones were selected.

Identification of hydrolytic bacterial strains using MIDI System through fatty acids methyl ester (FAME) profiling: Putative bacterial isolates recovered from soybean waste were characterized on the basis of extraction of whole-cell fatty acids methyl esters (FAMEs) profiling for bacterial identity. The whole-cell fatty acids of the bacterial isolates were derivatized to methyl esters and analyzed by gas chromatography (GC) using the Sherlock microbial identification system (MIDI, Inc, Newark, De, USA). The procedures and protocols used for growing the cultures and instrument specifications have been described by Sasser (1990). The fatty acids from bacterial isolates grown on tryptic soya agar for 24 h at 28 °C were extracted by saponification in dilute sodium hydroxide (15 % wt/vol) and methanol solution (50 % vol/vol), followed by derivatization with dilute hydrochloric acid and methanol solution to give methyl esters, and extracted with an organic solvent consisting of hexane and methyl tert-butyl ether. The resulting extract was analyzed by GC (Agilent Technologies, mode 7890A) using FID, HP-ULTRA 2 fused silica capillary column of 25 m x 0.2 mm x 0.33 µm with hydrogen as the carrier gas, nitrogen as the "make up" gas, and air to support the flame. The GC oven temperature was programmed from 17 to 270 °C at 50 °C increase per minute with 2 min hold at 300 °C at 21.3 psi. The fatty acids were identified and quantified by comparison with the retention times and peak areas obtained for the authentic standards mixture. Fatty acid profiles were compared with the Sherlock bacterial fatty acid reference library RTSBA6 of MIDI (Sasser, 1990).

Isolation of hydrolytic bacteria in soybean waste amended with wheat straw and cow dung

Based on GC-FAME, almost all the *Bacillus* isolates showed fatty acid as i-15:0 was the most abundant in the lipid profile compared to the Microbial Identification System (MIS) database, RTSBA6 (Microbial ID Inc. Newark, DE, USA) (Sasser, 1990) (Table 4 and 5). Many strains were not recognized by the database (identification similarity index < 0.200) or were identified with a low identification similarity index (0.3.00).

***Proteolytic isolates*:** A total of six isolates recovered from soybean wastes (hulls and dust) amended with wheat straw and cow dung were found to be proteins degrading. Most of the isolates were fast growing, gram +ve, possibly *Bacillus* sp. However, based on FAME, most of the isolates showed low response to match with the MIDI library and can be validated using molecular tools. One isolate, *Bacillus-alcalophilus* (FDD-P2) was characterized on the basis of fatty acids analyses carried out on MIDI System. The isolation of *Bacillus* has also been reported by other workers from industrial waste degradation materials other than soybean (Rastogi *et al.,* 2009; Tai *et al.,* 2004; Lee *et al.,* 2008). To date, a large number of alkaliphilic *Bacillus* strains have been isolated for industrial applications (Fritze *et al*., 1990; Khyami-Horani 1996). In most of the studies *Bacillus* sp. is designated as an alcalophilic which produced extremely thermostable alkaline protease which has various industrial applications, such as laundry detergents, leather preparation, protein recovery or solubilization. Ghanem *et al*. (2000) isolated *Bacillus alcalophilus*, an extremophilic bacterium from mangrove detritus, produced an extracellular alkaline-thermostable lipase.

***Amylolytic isolates*:** Among five isolates recovered on starch agar medium, three namely *Photorhabdus luminescens* (FDD-A1), *Bacillus alcalophilus* (FEDH-A1) and *Burkholderia gladioli* were identified as amylolytic based on differences in predominant fatty acids, rest two isolates did not match with the library and hence could be new or re-extraction of FAME is required for further confirmation. *Photorhabdus luminescens* was also found to possess proteolytic characteristics. The bacterium *Photorhabdus luminescens* strain was mainly associated (mutualistic) with entomopathogenic nematodes, which invade insect larvae and release the bacteria from their intestine killing them through the action of toxin complexes (Lang *et al.*, 2010). *Photorhabdus* lives in the guts of nematode worms which survive by hunting down insect larvae that live in the soil and on the stems and leaves of plant.

Apart from inbuilt insecticides, amongst the substances produced by *Photorhabdus* are also antibacterial agents that suppress the growth of other microbial species within the insect carcass, leaving *Photorhabdus* and its host nematodes to feed, grow and multiply unchallenged. It means, besides hydrolytic action, amylase producing enzyme which helps in utilization of starch and catalyses in to sugar molecules through amylolytic activity of strains is also involved. *Burkholderia gladioli,* a species of aerobic, gram –ve, rod-shaped bacteria formerly known as *Pseudomonas marginata*, causes disease in both humans and plants can also live in symbiosis with plants and fungi and is found in soil, water, the rhizosphere, and in many animals (Stoyanova *et al.*, 2007). Shimosaka *et al.* (2001) reported some strains, identified as *Burkholderia gladioli* strain CHB101 which produced three kinds of enzymes (two chitinases and one chitosanase) responsible for the degradation of chitinous compounds with a wide degree of acetylation (D.A. 0-100 %).

Lipolytic isolates: A total of four isolates were found to possess lipolytic properties on lipase agar medium. Of these, two isolates were found to be from genus *Bacillus and* characterized as *Bacillus clausii* and *B. alcalophilus,* based on higher similarity index, matched with the library and hence can be validated further using molecular techniques using16SrRNA gene sequencing approach. Rest two isolates showed low response could be new or could be reattempted for FAME analyses. Present report is in agreement with Kubo *et al*. (1994) who isolated and identified *Bacilus circulans* and *B. stearothermophilus*, which could degrade soybean wastes efficiently. These two strains secreted thermostable proteases into the medium and could digest soybean lees rapidly and completely at 50 °C. Initially, the soybean lees were degraded to proteins in approximately 20 h by these two strains, after which the concentrations of peptides in the medium gradually increased. The degraded products from soybean lees contained abundant nitrogen compounds, such as peptides, amino acids and amides.

Cellulolytic isolates: Out of three isolates recovered on CMC agar medium showing perceptible zone on congored overlaid drops, only one, namely *Pantoea agglomerans*-GC subgroup C (Enterobacter) showed a match in the MIDI library and remaining two did not match, hence could be novel and can be validated further using molecular tools. Present report of *Pantoea agglomerans* was supported by the work of Pepi *et al*. (2010) who isolated three strains of *Pantoea* and *Serratia* sp. from olive mill waste (OMW) and were found to degrade tannic acid utilizing it as sole carbon and energy source. The highest tannase activity was pointed out in the *Pantoea sp*. strain 2AT2, and growth tests showed maximum degradation rates of tannic acid within 6 h and a complete depletion in 24 h for all isolates. This was the first evidence of bacterial strains able to degrade tannic acid isolated from OMW. Similarly, Rezzonico *et al*. (2009) also reported *Pantoea agglomerans* strains which were most

Table 4. Details of potential hydrolytic bacterial isolates recovered from soybean waste amended with wheat straw and cow dung during decomposition under *in vivo* conditions at moisture content 40 ± 5%

Treatment	**Bacterial isolates**			
	Proteolytic (casein agar medium)	**Amylolytic (starch agar)**	**Cellulolytic (CMC medium)**	**Lipolytic (Lipase agar)**
Hulls (V) + wheat straw + cow dung (**1:4:0.5**)	FDH-P1	-	FDH-C1	FDH-L1
Dust (V) + wheat straw + cow dung (**1:4:0.5**)	FDD-P1	-	-	FDD-L1
Dust + hulls (P) + wheat straw + cow dung (**1:4:0.5**)	FEDH-P1	FEDH-A1	-	-
Dust (P) + wheat straw + cow dung (**1:4:0.5**)	FED-P1	FED-A1	FED-C1	-
Hulls (V) + cow dung (10:1)	-	FDH-A1	FDH-C2	FDH-L2
Dust (V) + cow dung (10:1)	FDD-P2	FDD-A1	-	-
[Dust + hulls (P)] + cow dung (10:1)	-	-	-	-
Dust (P) + Cow dung (10:1)	FED-P2	FED-A2	-	FED-L1

V- Vippy Industry food grade; P- Premier Industry feed grade

promising biocontrol agents for a variety of bacterial and fungal plant diseases, particularly fire blight of apple and pear. However, commercial registration of *P. agglomerans* biocontrol products is hampered because this species is currently listed as a biosafety level 2 (BL2) organism due to clinical reports as an opportunistic human pathogen.

From the present study, it is concluded that decomposition of soybean processing dust and hulls have predominance of hydrolytic bacterial strains such as proteolytic (*Bacillus alcalophilus*), amylolytic (*Bacillus alcalophilus*, *Bukholderia gladioli, Photorhabdus luminescens*), cellulolytic (Pantoea agglomerans), lipolytic (*Bacillus clausii, Bacillus alcalophilus*). These strains can be further validated using sequencing of 16SrRNA technique to exploit further for decomposition of soybean wastes eventually use as soil conditioner for enhanced growth and mineral nutrition of soybean.

Table 5. Identification of hydrolytic bacterial strains through FAME extraction using GC-MIDI system (based on similarity index more than 0.3.00)

Hydrolytic bacteria	Isolate	Most predominant fatty acids	Strain identified
Proteolytic	FDH-P1 FDD-P1	Total low response in matching fatty acids	
	FDD-P2	15:0 iso (15.1%), 20:2 w6,9c (7.8 %), 16:0 (15.3 %)	*Bacillus alcalophilus*
	FEDH-P1 FED-P1 FED-P2	Total low response in matching fatty acids	
Amylolytic	FEDH-A1	Total low response in matching fatty acids	
	FED-A1	15:0 iso (18.7 %), 16:0 (16.5 %), 17:0 cyclo (3.3 %)	*Bacillus alcalophilus* (some 48h)
	FDH-A1	16:0 (23.1 %), 17:0 cyclo (11.5 %) 18:0 (3 %)	*Burkholderia gladioli*-GC subgroup B)
	FDD-A1	16:0 (25.1 %), 17:0 cyclo (8 %), 15:iso (6.2 %)	*Photorhabdus luminescens* (Xenorhabdus)
	FED-A2	Total low response in matching fatty acids	
Cellulolytic	FDH-C1 FED-C1	Total low response in matching fatty acids	
	FDH-C2	16:0 (24.8 %), 17:0 cyclo (10 %) 12:0 (6.4 %)	*Pantoea agglomerans*-GC subgroup C (Enterobacter)
Lipolytic	FDH-L2 FDD-L1	Total low response in matching fatty acids	
	FDH-L1	15:0 (38.4 %), 15 ante iso (22.6 %) 16:0 (8.3 %)	*Bacillus clausii*
	FED-L1	15:0 ante iso (24.6 %), 16:0 (13.1 %), 15:0 iso (14 %).	*Bacillus alcalophilus* (some 48h)

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Ansari M M and Gupta G K. 1999. Epidemiological studies of foliar diseases of soybean in Malwa plateau of India. Proceedings, World Soybean Research Conference VI, Aug 4-7, 1999, Chicago, Illinois, USA, 611p. **(Symposium/ Conf./Workshop)**

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