CHAPTER II

LITERATURE REVIEW

The common bean (*Phaseolus vulgaris* L.) is one of the most popular species of grain legume grown in Latin America and the Caribbean. The protein content of the bean seeds is 25 percent and consequently, this crop plays a major role as a source of protein in the daily diet (Harmandez, 1993). It is extremely versatile crop and varieties of its genotypes are cultivated in numerous different geographical areas. Its agricultural significance is also rising in Thailand, where some varieties of this species have recently been introduced for production of dry beans (*Phaseolus vulgaris* L.) in the highlands surrounding Chiang Mai (Henson, 1993).

2.1 Climatic Effect on Red Kidney Bean Yield

Common beans are a warm season crop and they are grown in a wide range of climates. Yields are usually higher in temperate than in tropical zones, but in the temperate areas they are grown in the warm season. Laing *et al.* (1984) showed that approximately 80 percent of all bean production in Latin America was in the regions where the mean temperature during the growing season was between 18 and 25 °C. Major production occurs in areas where the temperature is around 21°C. The optimum temperature range for germination of common bean is 20 to 30°C (Scully and Waines, 1987). In general, high temperature (>30°C) during flowering causes dropping of buds and flowers, which reduces the yield (Wallace, 1980). According to Laing *et al.* (1984) yield reductions below or above the optimum (21°C) were related to plant mortality (which varied substantially at high temperatures; Ishag and Agoub, 1974), reduce photosynthesis led to failure of flowers to produce mature pods. Abscission of flowers and failure to set and fill pods are probably the greatest constraints to bean yields. Failure rates are commonly 50 to 70 percent of opened flowers and the proportion increases above 30/25 °C day/night (Kay, 1979 *Cited by* Norman *et al*., 1995).

Wallace (1980) distinguished a 'below optimum temperature response' in which flowering is delayed at temperatures cooler than the optimum, and an 'above optimum temperature response' in which an increase in temperature above the optimum delays flowering proportionately. In addition, a photoperiod response delays flowering as the effective daylength becomes longer (for this short day spices). The photoperiod response may be positioned at, below or above the optimum temperature for flowering. There are, furthermore, interaction between temperature and photoperiod which delay flowering as either factor deviates from the optimum for minimal days to flower.

The second climatic constraint to yield is water. Kay (1979) *Cited by* Norman *et al*. (1995) reported that bean growing areas have an annual rainfall of 500-1500 mm. The production in Columbia and East Africa was most successful where rainfall during the growing period was 300-400 mm and seed maturation occurs in dry weather.

2.2 Nitrogen Response in Red Kidney Bean

Nitrogen is a mobile and dynamic nutrient, and its fate is governed by several competing physical and biological factors that interact with each other and the environment overtime.

Westermann *et al.* (1981) reported that the symbiotic N_2 contributed up to 90 kg N ha^{-1} which was 40 to 50 percent of the total N found in bean plants near physiological maturity. The amount of symbiotic N_2 fixed decreased as the available soil N or fertilizer N increased, and increased as the N required by the individual cultivars increased. The response to N fertilization depended upon the cultivar, as well as on the N available from soil sources. Measured fertilizer N recovered ranged from 7 to 33 percent. An average of 52 percent of the total N uptake near physiological maturity was taken up after maximum symbiotic N_2 (acetylene reduction method) rate occurred; while the seed contained an average of 60 percent of the total N uptake. A low N fertilization rate (< 50 kg N ha^{-1}) when the soil N_2 was low (< 50 kg N ha^{-1}) ensured an early vigorous plant growth but did not always increase seed yields. Higher N fertilization rates may be required for soils with lower amount of mineralizable N.

Edje *et al.* (1975) studied the effects of six levels of nitrogen (0, 40, 80, 120, 160 and 200 kg ha^{-1}) on determinate dry bean yields for 2 years. It was found that *Phaseolus* bean seed yield increased significantly in both years with increasing rate of nitrogen. Average yields were 2150, 2704, 3048, 3147, 3366 and 3779 kg ha^{-1}, respectively, for the six rates of N indicating that higher rates of N might have produced greater seed yields. Yield components (yield/plant, pods/plant, seed size) also increased with increasing nitrogen.

Common bean is capable of fixing large amounts of atmospheric N_2 under certain condition, but usually some mineral N is needed to achieve substantial yield under prevailing cropping system. However, nitrogen fertilizer affects nodulation of bean plants and therefore the usually recommended rate of 40 to 60 kg N ha^{-1} would suppress N_2 fixation. On the other hand, low rates have been shown to enhance nodule formation and function but are not sufficient to achieve maximum yields (Tsai *et al.*, 1993).

Afza *et al.* (1987) indicated that it was possible to apply small amounts of soil or foliar N fertilizer, which might increase yield without reducing the amount of nitrogen fixed. This field experiment was performed by applying 20 kg N ha^{-1} as starter and additional 40 kg N ha^{-1} as soil and/or foliar fertilizer to soybean. Additional 40 kg N ha^{-1} soil or foliar fertilizer application increased the seed yield significantly without reducing the amount of N_2 fixed. However, additional fertilizer with 40+40 kg N ha^{-1} reduced nitrogen fixation (Boote *et al.*, 1978; Poole *et al.*, 1983).

Dart (1974) studied on *Vigna sinensis* and reported that there was usually a period of nitrogen hungry during seeding development before an effective symbiosis could establish.

Both the formation of nodules and activity of enzyme responsible for nitrogen fixation (nitrogenase) are strongly reduced in the presence of moderate or high levels of combined nitrogen. However, small amounts of fertilizer nitrogen are often beneficial to overall plant growth and nitrogen fixation. It is assumed that this occurs because the early nitrogen deficiency is overcome, thereby allowing earlier nodule functioning and the development of more nodule tissue. Muller (1993) reported that the low N application was to stimulate N_2 fixation by improving early plant growth until N_2 fixation provided adequate N for plant growth and development. On the other hand, fertilizer rates exceeding those exerting a starter N effect, generally have reduced nodulation and N_2-fixation.

Beard *et al.* (1971) showed that the number of nodules were reduced when nitrogen was added at planting time but not when nitrogen was applied at the flowering stages. The adequate concentration of nitrogen nutrient levels of common bean (*Phaseolus vulgaris*) at early flowering stage on the uppermost blade was 15 to 54 g kg^{-1} and at the peak harvest, when analyzed from common bean pods, the adequate concentration was 31 g kg^{-1} (Fageria *et al.*, 1991).

2.3 Nitrogen Fixation in the Red Kidney Bean

Common bean is generally considered to be weak in N_2 fixation and shows a variable response to inoculation (Vincent, 1974). Acetylene reduction rates were estimated for 18 dry bean cultivars at Kimberly, Idaho (Westermann and Kolar, 1978). The calculated nitrogen fixation was about 10 kg N ha^{-1}, a small part of the total N uptake of 150 to 400 kg ha^{-1}.

Bliss (1993) reported that locally adapted bean cultivars were able to fix at least 50 kg N ha^{-1} which was 40-50 percent of the plant N from fixation. When these levels were attained and other factors were not greatly limiting, grain yields from 1 to 1.5 t ha^{-1} were produced, as would be expected based on N composition of grain and straw.

Piha and Munn (1987) studied on nitrogen fixation capacity of field grown bean compared to other grain legumes and found that the amount of N fixed by a legume depended on the efficiency of the symbiosis, but was limited genetically by the growth of the host and environmental by the N status of the soil. The plant's capacity to accumulate N when adequately fertilized with combined N probably represents an upper limit to N_2-fixation capacity. When *P. vulgaris* was fertilized with ammonium nitrate it accumulated less N than cowpea and soybean. Furthermore, during vegetative growth, N_2-fixation by early maturing bean was generally inadequate. For later maturing bean the symbiosis performed moderately well. Nitrogen fixation was more favorable during the later part of the growth cycle of a legume than it was during early growth when compared to nitrate assimilation.

Recent studies indicated that foliar N application during seed development might be beneficial to some legumes (Garcia *et al.*, 1976; Neumnn *et al.*, 1979), however, no consistent soybean (*Glycine max* (L.) Merr.) yield increase had occurred for several N application methods and N sources (Welch *et al.*, 1973). In contrast, beans responded to N fertilization (Edje *et al.*, 1975). This indicated that their symbiotic N_2 fixation process generally did not provide sufficient N for maximum yields.

Tsai *et al.* (1993) reported that, although common bean (*Phaseolus vulgaris* L.) had good potential for N_2 fixation, some additional N provided through fertilizer was usually required for a maximum yield. The overall average nodule number and weight increased under high fertility levels. At low N application, nitrogen had a synergistic effect on N_2 fixation, by stimulating nodule formation, nitrogenase activity and plant growth. At high fertility and at highest N rate (120 mg kg^{-1} soil), the stimulatory effect of N fertilizer on N_2 fixation was still observed, increasing the amounts of N_2 fixed from 88 up to 375 mg N $plant^{-1}$. These results indicated that a suitable balance of soil nutrients was essential to obtain high N_2 fixation rate and yield in common bean.

Sangchan (1993) reported that nitrogen application of 70 kg N ha^{-1} provided nitrogen fixation of 38 kg N ha^{-1} which was 56 percent of total N accumulated in plant. The nitrogen application, on the other hand, depressed nitrogen fixation. The effect was small at full bloom (R_2), but became more severe from full pod set (R_4) to full seed growth (R_6), when the bean with nitrogen fertilizer derived only half of its nitrogen from fixation compared with more than 80 percent when nitrogen was not applied.

Hardarson *et al.* (1993) estimated the proportion (Pfix) and amount of N_2 fixed in common bean and found that, N_2 fixed varied from 0 to 73 percent and amount of N_2 fixed ranged from 0 to 125 kg N ha^{-1}. The amount of N_2 fixed by different leguminous crops (Table 2.1) were reported by Wani and Lee (1992), Peoples and Crasswell (1992).

Table 2.1 Example of estimates of nitrogen fixed by some legumes

Crop	Nitrogen fixed (kg ha^{-1})
Alfafa	100-300
Black gram	119-140
Clover	100-150
Chickpea	23-97
Cluster bean	37-196
Common bean	3-57
Cowpea	9-125
Groundnut	27-206
Lentil	35-100
Green gram	50-66
Pigeonpea	4-200
Rice bean	32- 97
Soybean	49-450
Peas	46
Fenugreek	44

Sources : Derived from Wani and Lee (1992), Peoples and Crasswell (1992).

Watanabe *et al.* (1983) reportedly a positive correlation between yield and N accumulation. Supplemental N increased both carbon and nitrogen accumulation. Symbiotic N_2 fixation, however, was inhibited by N fertilizer.

Therefore, the absorption of supplemental N might be negated by the depression of N_2 fixation.

Under tropical environments in Brazil, common bean (*Phaseolus vulgaris* L.) was able to fix 20-60 kg N ha^{-1}, but this amount is inadequate to meet the N requirement for economically attractive seed yield. When the plant is supplemented with N fertilizer, N_2 fixation by *Rhizobium* can be suppressed even at low rate of N. Watanabe (1983) pointed out that generally, N_2 fixation decreased as N application increased. However, when small amounts of N were applied at planting (e.g. 50 kg N ha^{-1}), N_2 fixation increased during the latter growth stage, a period when N fertilizer in the soil had already been exhausted. Because the N_2 fixation activity of the root nodules was small during the plant's early growth stages, basal N application can be used to promote growth. During the plant's later growth stages, N_2 fixation becomes active even with a small amounts of basal N. Thus, larger quantities of N can be accumulated during the growth cycle. Consequently, application of 20 to 30 kg N ha^{-1} at planting was recommended in Hokkaido (Silva *et al.*, 1993).

Watanabe *et al.* (1983) reported that soybean's N demand during ripening stage, and its low response to basal fertilizer, might be best resolved by applying supplemental N after flowering. This practice could increase carbon assimilation by augmenting photosynthesis. It might also increase nitrogen assimilation to meet N requirement for seed development. Application of supplemental N after flowering had been tried before, but the results were conflicting.

Westermann *et al.* (1981) concluded that beans (*Phaseolus vulgaris* L.) utilized inorganic soil N or applied fertilizer N and N_2 fixed by symbiotic relationship with *Rhizobium phaseoli.* Both the inorganic and symbiotic N source seemed necessary for maximum yield of seed legumes.

Tiyawalee *et al.* (1978) studied on the effect of nitrogen fertilizer and rhizobium on common bean and showed that rhizobial inoculation could increase bean yield about 39 percent, while nitrogen fertilizer at 12.5 kg N ha^{-1} the increase was only 15 percent. The contribution of rhizobial and nitrogen fertilizer provided 55 percent yield increment.

Westermann and Kolar (1978) studied the response of bean to N fertilization under fields conditions and indicated that the different Rhizobia-cultivar relationship or symbiotic N_2 fixation limitations due to the cultivars. Adams *et al.* (1985) reported that among major problems which limited response to inoculation in the field were due to variation in host genotype ability to fix N, lack of well tested strain of rhizobium for inoculation, soil acidity problems and host plant nutrition. According to Isoi and Yoshida (1991) three factors contributing to low nitrogen fixation in common bean were: (1) delay of nodule appearance on root system, (2) insufficient nodule mass on root system and (3) ineffectiveness of nodular formed on root system.

There was variation in the genetic capacity of different species to grow and fix N_2 under the same environmental conditions as shown in Table 2.2 (Peoples *et al.*, 1995).

Table 2.2 Comparisons of the N_2-fixation capacities of different species of food legumes

Location and species	N yield (kg N ha^{-1})	N_2 fixation	
		P fix (%)	Amount (kg N ha^{-1})
Thailand[a]			
Green gram	74	89	66
Black gram	125	95	119
Soybean	65	87	57
Australia[a]			
Black gram	56	37	21
Soybean	282	90	254
Pigeon pea	36	44	16
Hawaii[a]			
Soybean	241	69	166
Common bean	142	16	23

[a] Peoples *et al.* (1994); George and Singleton (1992).

2.4 Other Essential Elements Necessary for N-Fixation

Mineral nutrient deficiencies can limit nitrogen fixation by legume *Rhizobium* symbiosis in many agricultural soils and as a result seriously depress potential yields (Marchner, 1986). Giller *et al.* (1991) concluded that several of the nutrients essential for growth of plants or bacteria played specific role in nodulation and/or N_2-fixation. Molybdenum deserves special consideration here as it is the major element required in plants for N_2-fixation. In fact, molybdenum is a constituent of both the enzymes nitrate reductase, required for assimilation of nitrate from the soil and nitrogenase. So molybdenum deficiency is manifested as a deficiency of plant N. If a symbiosis is established, unusual proliferation of nodules is often absented when legumes are deficient in molybdenum, presumably in response to the ensuing N deficiency of the plant. Phosphorus plays a key role in building up and maintenance of soil productivity by legumes through its effect on host plant growth and its specific effect on rhizobium growth, survival, and nodulation capability (Giller *et al.*, 1991). Increased P supply showed increase nodule number and weight, or rate of N_2 fixation in a number of leguminous crops, i.e. common bean (Graham and Rosas, 1979; Ssali and Kenya, 1986) and chick pea (Shukla and Yadav, 1982). Phosphorus supply has a stimulatory effect on N_2 fixation, although in most cases the enhancement can be attributed to improved host plant growth, and to the interaction of this with N_2 fixation (Mclaughlin *et al.*, 1990). Phosphorus deficiency is a factor commonly restricting the realization of the potential of N_2-fixation by legumes mainly through yield reduction but also percent N lowered in the tissue. The reduction of the proportion of N derived from fertilizer under severe P deficiency were also reported (Thomas, 1995).

Pandey and Mcintosh (1988) concluded that P could interact strongly with other plant nutrients. It can increase efficiency of symbiosis and N_2 fixation by grain legumes. The N concentration in the tops of P deficient legumes is usually low and good correlation between P and N concentrations in tropical grain legumes have been established. This effect may be attributed to at least four possible functions: root development, nodulation, nodule efficiency, and plant metabolism.

Giller *et al.* (1991) reported that, cobalt is unusual in that it is used in the cobamide electron transport pathways of rhizobia and is thus essential for N_2 fixation in legumes. In addition, copper deficiency also reduces N_2 fixation.

2.5 Contribution of Rhizobium to Yield Increment

Schroder *et al.* (1992) found that in Puerto Rico, the inoculated treatments yielded 112 kg more dry bean per ha than that of the uninoculated treatments, but this increase was not statistically significant. Nodulation could be improved by inoculation, but this early response was not accompanied by significant yield increases.

Duong *et al.* (1984) reported that soybean inoculated with an granular inoculant increased yields from less than 500 kg ha^{-1} to nearly 2700 kg ha^{-1}. In subsequent trial yields of inoculated soybeans (< 2800 kg ha^{-1}) were ten times as high as those without either inoculation or nitrogen fertilizer and over twice as high as uninoculated plants which received 80 kg N ha^{-1} of urea (Table 2.3).

Table 2.3 Response of soybean to N fertilizer as urea on inoculation with rhizobium in acid sulphate soils (pH 4.5-5.0) of the Mekong Delta

Treatment (kg N ha^{-1})	N in harvested grain (kg/ha)	N in plant remains at maturity (kg/ha)	Grain protein (%)	Grain yield (kg/ha)
0	17	1	36	290
20	19	-	31	385
40	29	8	26	680
60	24	8	28	780
80	32	14	28	1140
Inoculated + ON	185	15	40	2870

Source : Adapted from Duong *et al.* (1984).

In Brazil, Bliss (1990) indicated that inoculation treatments were not significantly different from the uninoculated control, although the mean values of the inoculated treatments were often higher, particularly for grain yield. Duque *et al.* (1985) were able to obtain the response of inoculation but only in certain varieties. Rerkasem and Rerkasem (1993) showed that inoculated with appropriate bacteria, the red kidney bean could be effectively nodulated by the bacteria that had become naturalized in the soil. At Chang Kian station of CMU (elevation 1,200 m), an uninoculated crop of red kidney bean cv. Mokcham took up 90 kg N ha^{-1} to give seed yield of 1.754 t ha^{-1}. Giller *et al.* (1991) indicated that inoculation was most likely to be necessary when legumes were introduced into new areas, although the need for inoculation would of course be conditioned by the requirements of the introduced legume for its specific strains of rhizobia. If the introduced legume crop could nodulate effectively with rhizobia which are present in the soil in sufficient numbers, then inoculation may not be necessary.

Environmental factors affect results obtained by inoculation under field conditions. Results from world wide legume inoculation trials indicated that *P. vulgaris* inoculation generally did not increase yield (Bohlool, 1988).

Major factors contribution to inoculation failure

1. Strain competition

One of the major factor of inoculation failure in Puerto Rico was competition from local adapted strains that had been shown to occupy most of the nodules (Velazquez *et al*., 1988). In Argentina, *P. vulgaris* plants were spontaneously but poorly nodulated, and their moderate grain yield did not generally show a positive response to rhizobium inoculation (Pachelo Basurco *et al*., 1990 *cited by* Schroder *et al*., 1992). Neves *et al*. (1985) claimed that certain strains of rhizobia could enhance the partitioning of fixed nitrogen to seeds, and thereby lead to increased yield. Jaichoob (1994) concluded that efficiency of *rhizobium* strain was possible when *rhizobium* strain was fit with bean varieties. Apart from this, soil condition was an important factor to rhizobium's survival.

2. Nitrogen supply

Several environmental factors affect field nodulation and nitrogen fixation. The effect of additional N fertilizer had been evaluated by Mangual-

Crespo *et al.* (1987), and the results showed that small additional could increase yields and stimulate nodulation. Similar results had been obtained in Kenya, where starter dose of 20 kg N ha^{-1} had small effect on nodulation (Ssali and Kenya, 1982). Abaidoo *et al.* (1990) showed that the application of N fertilizer reduced the nodule mass in *P. vulgaris* to a greater extent than in soybean (*Glycine max*).

3. Soil acidity factors

In tropics, the acid soil complex affects nodulation and nitrogen fixation (Andrew, 1978 *cited by* Schroder *et al.* 1992). Graham *et al.* (1982) found that rhizobia strains varied in terms of their tolerance to acidity. Rerkasem and Rerkasem (1993) reported that, soil acidity constrainted grain legume production in tropical soil by limiting rhizobium survival and reducing nodulation. Franco and Munns (1982) found that the solution culture having pH 4.5 to 5.5 did not affect nodule growth and nitrogenase activity. However, a decrease in pH from 5.5 to 5.0 resulted in a decrease in the number of nodules formed per plant. Munn and Fox (1977) studied the comparative lime requirements in tropical and temperate, and found that *P. vulgaris* had higher requirements than the other legumes. Buerkert *et al.* (1990) showed that liming acid soils in southern Mexico increased nodulation and nitrogen fixation, resulting in better establishment and increasing of pod number per plant, seed number per pod, seed weight and seed yield. Graham *et al.* (1982) classified *rhizobial* 55 strains on media which provided a pH, Mn and Al stress. One strain classified as tolerant, survived better than a sensitive one when inoculated into acid soil adjusted to pH 4.15, 4.5 and 4.9. Tolerant strain also improved the yield of beans at pH 4.5, 4.75 and 5.2.

4. Plant nutrient deficiencies

Soils in the major bean producing region of Latin America are often deficient in P. Since nodules were extremely strong sinks for P, thus nitrogen fixation was limited by P (Graham and Rosas, 1979). Field experiments in Kenya indicated that nodule mass, dry matter yield, N yield and nitrogen fixation increased by the application of P (Ssali and Kenya, 1982). A green house experiment conducted by Bonetti *et al.* (1984) showed that nodulation and plant weight increased under increasing rate of P when water tension was low. Loneragan and Dawling (1958) found that the requirement of calcium was definite but low (approximately 10 μM) in simple media. Nevertheless, it had been demonstrated that calcium could limit nodulation of *Trifolium subterranean* by restricting growth of rhizobia in rhizosphere.