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Effect of Bradyrhizobium japonicum Population on Nodulation, Nitrogen Fixation and Dry Matter Yield on Soybean (Glycine max L Merrill) in Some Ghanaian Soils

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Abstract
In as much as the noted ability of the promiscuous TGx soybean varieties developed by the International Institute of Tropical Agriculture in Ibadan to nodulate profusely with indigenous bradyrhizobia confers advantages where lack of fertilizers and suitable inoculants act as disincentives to growing the crop, the ability of these nodules to fix nitrogen should be even more important. Selection for promiscuous nodulators in different soils must therefore go hand in hand with their nitrogen fixing abilities. The symbiotic performance of promiscuous soybeans depends upon the population size and survival of bradyrhizobia on the field. A pot experiment was conducted in the greenhouse within the Geography Department in University of Ghana-Legon using soils collected from ten farmer's field located in one Agro Ecological Zone (Coastal savanna) to determine nodulation, nitrogen fixation and dry matter yield by indigenous bradyrhizobia in five Ghanaian soils using three promiscuous soybean varieties and one American type, Bragg, a non promiscuous variety. Nodulation by the three promiscuous soybean varieties (Bengbie [TGx 536-2D], TGx 1835-10E, and TGx 1830-20E) ranged between 21 to 25 and the non-promiscuous variety (Bragg) recorded 13. Hake soil series which contains the highest bradyrhizobia cell count (6.0 x 10^3 cellgsoil-^{1} also produced the highest number of nodules 22. Hake again produced the highest nodule dry weight, (176.0mg), shoot dry weight (3.85g), Total nitrogen (N) (106.0mgN plant^{-1}), Total N fixed (88.9mgN plant^{-1}) and %N fixed (69%). TGx 1830-20E also maintain its superiority in nodule dry weight (220.0mg plant^{-1}) production, Total N (105.3mgN plant^{-1}), Total N fixed (88.5mgN plant-1) and % N fixed (83.43). Bragg performed poorly in terms of nodule dry weight (67.0mg plant-1), Total N (83.8mg plant^{-1}), Total N fixed (67.0mg plant^{-1}) and %N fixed (78.05). The Most Probable Number (MPN) counts on sand grown soybean indicated 6000, 5300, 4700, 3600 and 3200 native *B. japonicum* cells g-^{1} in Hake, Goi, Simpa, Agawtaw and Prampram soils respectively. The nodulation, N content and dry matter yield results showed that Ghanaian soils contain effective bradyrhizobia capable of nodulating soybean and N_2 fixation rendering inoculation unnecessary.
Keywords: Agrecological zone. Effective bradyrhizobia. Nitrogen fixation. Nodulation Promiscuous soybean

INTRODUCTION

Soybean crops generally require large supplies of N to achieve their maximum yield potential (George & Singleton, 1992). Successful nodulation of these promiscuous soybeans depends on the presence of sufficient number of compatible and effective indigenous bradyrhizobia to achieve their maximum yield potential (George & Singleton, 1992).

In 1978, the International Institute of Tropical Agriculture (IITA) soybean program targeted the improvement of Biological Nitrogen Fixation (BNF) for soybean through a breeding program to develop promiscuous soybean varieties known as Tropical Glycine Cross (TGx) that nodulate freely with indigenous soil bradyrhizobia thus eliminating the need for inoculation. Nodulation of soybean by effective bradyrhizobia can result in substantial amount of N being fixed by the symbiosis (Henson & Heibel, 1884; Legg and Sleger, 1975). However responses of these promiscuous soybeans to inoculation have been reported in Nigerian soils (Okereke and Eaglesham, 1993) and in Ghanaian soils Kumaga & Etu-Bonde (2000) indicating that either these soils lacked sufficient numbers of compatible and effective bradyrhizobia to infect nodulation on the host plant. Furthermore studies with these promiscuous soybean varieties have however shown considerable variability in the effectiveness and population of communities of indigenous bradyrhizobia in a given location (Sanginga *et al*., 1999; Fening & Danso, 2002). Sanginga *et al*., 1995 also found a direct relationship between bradyrhizobia cell count and promiscuous soybean response in the moist savanna of Nigeria. In most Ghanaian soils few bradyrhizobia strains were detected and they were not competitive or compatible enough with the legume host to form nodules unless these legume host were inoculated with effective bradyrhizobia (Owiredu and Danso,1987; Kumaga & Ofori, 2004). In this study, it is hypothesized that Ghanaian soils contain more indigenous bradyrhizobia capable of supporting nodulation and Nitrogen fixation.

Our present study was initiated to determine the effect of indigenous bradyrhizobia on nodulation, N fixation and dry matter yield of soybean in some Ghanaian soils.

MATERIALS AND METHODS

Soil analysis: Total soil N was estimated using the Kjedahl digestion method (Bremner, 1996). Available P and Calcium was also determined. Soil pH was determined on a 1:2 soil to water ratio.

Soil sampling: Ten different sites were chosen to cover the main agro-ecological zones and soil types of Ghana. All the sites have no recent history of soybean cultivation. At each site, twenty random sub-samples from the top 20cm were collected using a pick-axe and shovel and mixed thoroughly in a plastic bucket. The composite samples were placed in a new plastic bag and transported to the laboratory in an ice-cold box away from direct sunlight to reduce mortality of bacteria due to heating and ultra-violet light. Samples were kept in the store room of the laboratory and processed within two days of collection

Enumeration of rhizobia: The populations of rhizobia capable of nodulating soybean in the five soil series were enumerated by the Most Probable Number (MPN) method (Vincent, 1970) using Leonard jars (Somasegaran and Hoben, 1985). Soybean variety, TGx 1830-20E was used as the trap host. Clean seeds, tested and shown to be of high viability (99%) were surface sterilized in 70% alcohol for 3 minutes and 0.1% Mercuric Chloride for 3 minutes. The seeds were then rinsed in several changes of sterilized distilled water (Somasegaran and Hoben, 1994). Seeds were germinated on 1 % (w/v) water agar for three days until the cotyledons were 2 cm long. Three pre-germinated seedlings were planted into each jar that had been sterilized by autoclaving. Seedlings were thinned to two five days after planting. Ten-fold dilutions of each soil sample in four replicates per dilution were used to inoculate the sand in the jar, at 1ml per jar. The jars were arranged in randomized complete block design in the greenhouse with minimum day temperature of 30ºC and 23ºC night temperature. The plants were observed periodically and their nutrient solutions supplied when necessary. Nodulation was assessed after 28 days of plant growth and the Most Probable Numbers (MPN) of bradyrhizobia were calculated. (Vincent, 1970)

Greenhouse studies and plant analysis: Five types of soils used were classified as Goi (Gleyic arenosols), Agawtaw (Stagnic solenetz), Hake (Eutric cambisols), Prampram (Calcic vertisols) and Simpa (Ferric luvisols) series with textural classes as grey sand, grey loamy sand, clay loam, sandy clay and loamy sand respectively. No inoculant was applied in any of the soil series, a move to see which of the soybean varieties would be most compatible with the indigenous bradyrhizobia. Bengbie and Bragg obtained from Soil Research Institute-Kumasi, Ghana, and TGx 1830-20E, TGx 1835-10E also obtained from IITA Ibadan-Nigeria and the non nodulating soybean obtained from the seed store of Crop Science department, University of Ghana were used in the study. Soybean seeds were surface sterilized (Somasagaran and Hoben, 1994) pre-germinated on 1 % (w/v) water agar and incubated at 28^0C for 3 days. Four seeds were planted in plastic pots (18cm high, 15cm wide at the top and 12cm at the base) filled with 1.2kg of soil sieved through 2mm mesh sieve and later thinned to two. All the soils were fertilized with essential macro and micro nutrients except N (Owiredu & Danso, 1988). The experiment was conducted in the Green House within the Geography Department of University of Ghana-Legon. The experimental design used was Randomized Complete Block Design and each treatment was replicated four times with soils as the main block. The plants were watered daily with tap water. Six weeks after planting, shoots were decapitated and nodules were separated from roots and counted. The nodules were sundried and stored in serum bottles for bradyrhizobia strain identification. The shoots were oven-dried to a constant weight at 60-65^0C for 48 hours (Owiredu & Danso,1987) and Kheldahl N analysis (Bremner, 1965) was done on ground samples (<0.2mm).

Nitrogen fixation by TND is calculated as follows:

N_2 Fixation = N_{total} (fixing legume) - N_{total}(reference crop). (Rennie, 1984)

Percent $N_{2\ fixed}$ = % N in plant shoot/Total nitrogen x 100

Statistical Analysis: Genstat statistical software version 6.1 Genstat (2000) was used to analyze the data. Significant differences were assessed at 5% level. Mean separation was carried out by Least Significant Difference (LSD) procedure.

RESULTS

INSERT TABLES 1,2,3,4,5,6,7 AND 8 HERE IN THAT ORDER

The chemical analyses conducted on five soils show that all the soils were slightly acidic except Prampram which was neutral. Hake and Simpa having the highest available P with Prampram having the lowest P value. Prampram soil recorded the lowest %N value while all the rest of the soils have sub-optimal levels of %N (Table 1). The MPN tests indicated the presence of *Bradyrhizobium* sp. in all five soils with the numbers and ranking being as follows Hake ($6.0x10^3$/gsoil)>Goi ($5.3x10^2$/gsoil)>Simpa ($4.7x10^2$/gsoil)> Agawtaw ($3.6 x 10^2$/gsoil) > Prampram ($3.2 x10^2$/gsoil). This shows that soil has significant effect on the abundance of soybean bradyrhizobia. The result showed that the population of bradyrhizobia in these five soils were sufficient to support nodulation. Without even inoculation, there were significant (P<0.05) interactions between soybean variety and soil containing *Bradyrhizobium japonicum* for all the trait scored indicating that the differences among the four soybean varieties were independent of inoculation. Soil has a significant (P<0.05) effect on the abilities of the soybean varieties to form nodules. Thus nodulation by naturally occurring bradyrhizobial strains was highly variable in the five soils and nodule number ranged from 14 for Bengbie, TGx 1830-20E, TGx 1835-10E and Bragg in Prampram soil series to 22 in Hake soils. Varieties also influenced the number of nodules formed by native strains. As expected, the non-promiscuous variety Bragg formed the least number of nodules, an average, 13 nodules per plant in the five soils. Nodulation among the promiscuous lines were also statistically significant. The corresponding average values were, 20.50, 21.70 and 24.80 for the promiscuous varieties, Bengbie, TGx 1835-10E and TGx 1830-20E respectively. TGx 1830-20E soybean, even though it produced the highest average number of nodules performed poorly in Prampram soils (15 nodules) but recorded the highest value in Goi soil in terms of nodule number (31) (Table2).

Similarly, significant differences ($P<0.05$) occurred among the four soybean varieties with regards to nodule dry weight and with a significant soil x variety interaction (Table 3).

The ranking among varieties for nodule dry weight was similar to that for mean number of nodules. TGx 1830-20E appeared to nodulate better and with the highest nodule dry weight (220mg plant^{-1}) while Bragg still had the least mean nodule dry weight per plant (113.5mg plant^{-1}) in all the five soil series. Mean nodule dry weight values for the intermediates, plants grown on the Goi and Agawtaw soils (136 and 145mg plant^{-1} respectively) were statistically similar ($P<0.05$). While Hake soil series recorded the highest mean nodule dry weight, per plant (176mg plant^{-1}) for the soils in which nodulation occurred, Prampram produced the lowest mean nodule dry weight per plant (129.0mg plant^{-1}), a trend similar to that of the nodule number.

Significant ($P<0.05$) differences again existed among soybean varieties and soil for shoot dry matter yield except TGx 1830-20E and TGx 1835-10E which produced similar shoot dry matter yield (3.70 and 3.71g plant^{-1}) (Table 4). Bragg recorded quite a higher shoot dry matter yield (83.8g plant^{-1}) despite its fewer nodules. Prampram and Simpa soil series produced similar shoot dry matter yield with Agawtaw soil series producing the highest shoot dry matter yield indicating that there is no correlation between nodule number, nodule dry weight and shoot dry matter yield.

Although TGx 1830-20E overall accrued the highest plant N (mean,105.3 mg plant^{-1}), there were not much statistical difference ($P<0.05$) among the varieties with the the exception of the non-nodulating variety for which N accumulated (16.8 mg plant^{-1}) was lower than for any other variety. (Table 6). All the five soybean varieties produced the highest average N (106.0 mg plant-^{1}) in Hake soil series and differed significantly ($P<0.05$) from the rest of the four soils (Goi, Agawtaw, Prampram and Simpa) are statistically similar (67.7, 69.3, 76.2 and 81.0 mg plant^{-1} respectively).

Even though TGx 1830-20E fixed the highest N_2 (83.4mg plant^{-1}) no significant difference ($P<0.05$) existed among the four soybean varieties but these are statistically higher than Bragg (67.0 mg plant-^{1}) (Table 7). Interestingly Bragg fixed the highest total N (119 mg plant^{-1}) in the Hake soil series compared to other three promiscuous soybean varieties. Soil effect appeared more pronounced than the varietal influence on differences in the total N fixed by plants. Hake soil series again maintain the highest total N fixed value (1118.2 mg plant^{-1}) while Agawtaw soil series recorded the lowest total N fixed (65.2 mg plant^{-1}).

Apart from soybean variety, Bragg, no significant ($P<0.05$) difference existed among the three promiscuous soybean varieties (Bengbie, TGx 1830-20E and TGx 1835-10E) in terms of %N_2 fixation in the five soil series (Table 8). While there is significant difference ($P<0.05$) among Hake, Prampram and Goi, Simpa and Agawtaw were statistically similar ($P<0.05$). Hake still maintain its superiority in %N2 fixation recording 86.3% with the lowest value of %N2 being registered by Simpa (78.6%).

DISCUSSION

Nodulation is said to be favoured by low N, high available P and high pH in soils. The high P in Hake and Simpa soils perhaps promote profuse nodulation in these soils. Prampram soil having the lowest %N should have recorded the highest nodule number but failed to do so perhaps due to other soil factors or its low available P. All the soils produced nodules by soybean since none had extremely low pH.

Soil has a significant effect on the abundance of bradyrhizobia resulting in over 10^3 bradyrhizobia cells gsoil^{-1} indicating that virgin soils used in this study are capable of harboring enough bradyrhizobia cells for nodulating soybean varieties. Significant differences existing between soil and variety interaction means that the variable nodule numbers produced by the soybean varieties depended on the soil series to do so. It also means that soil characteristics and bradyrhizobia strains in the soil interacted among the soybean varieties to produce variable nodule numbers. The high variability in counts of bradyrhizobia found in Ghanaian soils is similar to those reported by Danso and Owiredu (1988a), Danso (1992) and Fening (1999). The findings of this study disagree with Singleton &Tavares (1986) who reported counts of indigenous bradyrhizobia ranging from zero to a few hundred cells per gram of soil in the tropical soils of Hawaii and Singleton et al.,(1992) who also reported that in a study of field population of bradyrhizobia, nearly half of 305 tropical soils samples of different origin were found to contain an average less than 100 cellsg-^{1} soil. Earlier literature report also indicated that bradyrhizobia population counts in some temperate soils often range in the millions per gram of soils (Mpepereki and Wollum, 1991) and are generally higher than those found in many tropical soils. Result from this study also contradicts the general observation cited above that rhizobia numbers tend to be quite low in many tropical soil environments but agree with earlier report by Fening (1999) that the coastal savanna zone harbored the greatest number of cowpea bradyrhizobia and are higher than those found in soils under semi deciduous forests. The results also agree with the trend Fening (1999) observed for cowpeas and raises the question of the relatedness of cowpea and soybean bradyrhizobia and its influence on soybean nodulation.

Nodulation was high in most of the soybean varieties perhaps due to the fact that bradyrhizobia counts are high in these soils. These findings also agree with Danso (1992) that a population range of 10^3 to10^4 bradyrhizobia per gram of soil should by most standards be adequate for high nodulation. Experiment with the American type soybean cultivars in most tropical soils had shown that these plants would not nodulate with the indigenous bradyrhizobia. lt is therefore not surprising that in the present study, the non-promiscuous cultivar Bragg nodulated poorly in all the five soils. This present study seems to agree with reports in the literature that non-promiscuous American soybean genotypes nodulate poorly in tropical soils (Pulver *et al*., 1978; Pulver *et al*., 1985; Ranga Rao *et al.,* 1982; Nangju 1980). In general, the entire promiscuous soybean lines nodulated faily well with indigenous bradyrhizobia and this is in agreement with work done by Singh and Rachie (1987). This also confirmed findings of Pulver *et al.,* (1982 and 1985) and Nangju (1980). The TGx varieties nodulated fairly well perhaps because they were purposely bred with genes for promiscuous nodulation incorporated into them by International Institute for Tropical Agriculture (IITA). This agrees with earlier report in the literature by Kueneman *et al*., (1984) & Pulver et al., (1982) who observed that all TGx lines tested formed many nodules on soybean grown in soils without inoculation compared to the American-type Bragg and Davis. The high nodulation in Bengbie soybean perhaps may be due to the fact that they were parental stock and had been used in the country for a long time for which reason their compatible bradyrhizobia were abundant. In this study TGx 1830-20E fixed 88.5mgN/plant and 83.43 % N/plant in all the five soils. This result contradicts Bezdicek *et al.,* (1978) which showed that soybeans are capable of fixing over 300mg N/plant when effective strains of bradyrhizobia are supplied in high numbers and the soil is also low in available nitrogen. Of the three promiscuous varieties (Bengbie, TGx 1830-20E and TGx 1835-10E) that accumulated higher N and produced higher shoot dry weights were also associated with higher nodulation and N2 fixation, suggesting that the higher N demand could have stimulated higher N2 fixation (Thies et al. 1991b). This result indicating no significant difference among the three promiscuous soybean varieties was perharps due to the fact that bradyrhizobia in these soils competed for nodule occupancy and nitrogen fixation since percent N fixation is the true revelation of the genetic constitution for N_2 fixation by soybean varieties. Besides the closeness in nitrogen fixation among the soybean varieties is an indication that the bradyrhizobia in these soils were effective and also there was host-strain compatibility. The findings of this study revealed that nodulation of both promiscuous and non-promiscuous soybean varieties were directly proportional to nodule formation, nodule dry weight, shoot dry matter yield, total nitrogen accumulation and total and percent nitrogen fixation hence if only substantial amount of competitive and compatible *Bradyrhizobium japonicum* can be located in many tropical soils more nitrogen will be fixed for soybean development and other crops such as maize as well.

CONCLUSION

It can be concluded that *Bradyrhizobium japonicum* in Ghanaian soils resulted in the formation of more nodules, nitrogen fixation and shoot dry weight of all the promiscuous soybean. Besides, soybean has a high potential for N_2 fixation judging from its high N accumulation from atmospheric N_2 The non-promiscuous variety, Bragg form fewer nodules, sub-optimal nitrogen fixation and shoot dry matter yield. Soil was an important determinant of how much nitrogen was fixed. The profuse nodulation in Hake soil resulted in high shoot dry weight, nitrogen acreation and high nitrogen fixation than the other soils.

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Table 1. Some physical and chemical properties of five soils used for growing uninoculated soybean

Soil Series	pH(in H_20)	% N	Total P($mgkg^{-1}$)	Available P($mgkg^{-1}$)	Calcium (Me/100)
Goi	6.4	0.50	24	5.72	2.0
Agawtaw	6.2	0.46	29	5.33	2.0
Hake	6.7	0.44	199	6.97	6.4
Prampram	7.2	0.31	152	4.55	6.8
Simpa	5.8	0.49	44	6.97	3.2

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Table 2: Mean nodule number per plant of five uninoculated soybean varieties in five different soils

	Soil series					
Varieties	Goi	Agawtaw	Hake	Prampram	Simpa	Mean
Bengbie	15.8	17.5	28.0	17.5	23.8	20.5
TGx 1830-20E	30.8	17.8	32.8	14.7	28.0	24.8
TGx 1835-10E	16.8	19.8	34.5	23.5	14.0	21.7
Bragg	13.5	13.3	11.8	11.8	11.8	12.7
Non-nod	0.0	0.0	0.0	0.0	0.0	0.0
Mean	15.4	13.7	21.7	13.5	15.5	-

LSD ($P < 0.05$); Soil =1.14, Variety =1.14, Soil ×Variety =2.55

Table 3: Mean nodule dry weight in mg per plant of five uninoculated soybean varieties in five different soils

	Soil series					
Varieties	Goi	Agawtaw	Hake	Prampram	Simpa	Mean
Bengbie	150.0	172.5	232.5	195.0	205.0	191.0
TGx 1830-20E	235.0	235.0	255.0	175.0	200.0	220.0
TGx 1835-10E	185.0	195.0	265.0	187.5	175.0	201.0
Bragg	112.5	122.5	127.5	87.5	117.5	113.5
Non-nod	0.0	0.0	0.0	0.0	0.0	0.0
Mean	136.5	145.0	176.0	129.0	139.5	-

LSD ($P < 0.05$); Soil =18.5, Variety =18.5, Soil ×Variety =41.3

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Table 4: Mean shoot dry weight in g per plant of five uninoculated soybean varieties in five different soils

	Soil series					
Varieties	Goi	Agawtaw	Hake	Prampram	Simpa	Mean
Bengbie	2.65	3.17	4.28	3.57	4.28	3.59
TGx 1830-20E	3.35	3.18	4.29	3.97	3.71	2.85
TGx 1835-10E	3.41	3.45	4.39	4.02	3.29	3.71
Bragg	2.51	2.81	4.36	2.71	2.86	3.05
Non-nod	1.73	1.80	1.92	1.91	1.99	0.90
Mean	2.73	2.88	3.88	3.23	3.23	-

LSD ($P < 0.05$); Soil =0.3, Variety =0.3, Soil ×Variety =0.7

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Table 5: %N determination of plant shoot per plant of five uninoculated soybean varieties in five different soils

	Soil series					
Varieties	Goi	Agawtaw	Hake	Prampram	Simpa	Mean
Bengbie	2.34	2.37	3.09	2.46	2.93	2.63
TGx 1830-20E	2.99	2.74	2.84	2.81	2.88	2.85
TGx 1835-10E	2.67	2.70	2.75	2.45	2.70	2.66
Bragg	2.77	2.74	3.12	2.54	2.34	2.70
Non-nod	0.87	0.92	0.89	0.81	0.99	0.90
Mean	2.33	2.29	2.54	2.21	2.37	-

LSD ($P < 0.05$); Soil =0.2, Variety =0.2, Soil ×Variety =0.5

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Table 6: Total N (mg plant-1) of five uninoculated soybean varieties in five different soils

	Soil series					
Varieties	Goi	Agawtaw	Hake	Prampram	Simpa	Mean
Bengbie	60.5	74.9	131.2	88.7	124.8	96.0
TGx 1830-20E	101.6	84.1	122.7	111.2	106.8	105.3
TGx 1835-10E	92.3	92.2	122.9	97.1	87.6	98.4
Bragg	69.7	78.1	136.2	68.3	66.5	83.8
Non-nod	14.7	17.1	17.1	15.8	19.5	16.8
Mean	`67.7	69.3	106.0	76.2	81.0	-

LSD ($P < 0.05$); Soil =11.26, Variety =11.26, Soil ×Variety =25.19

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Table7: Total N2 (mg plant-1) fixed per plant of five uninoculated soybean varieties in five different soils

	Soil series					
Varieties	Goi	Agawtaw	Hake	Prampram	Simpa	Mean
Bengbie	46.0	57.8	114.1	72.9	105.3	79.2
TGx 1830-20E	87.1	67.0	105.6	95.4	87.3	88.5
TGx 1835-10E	77.8	75.1	105.8	81.3	68.1	81.6
Bragg	55.2	61.0	119.2	52.5	47.0	67.0
Mean	80.7	65.2	111.2	75.5	76.9	-

LSD ($P < 0.05$); Soil =11.30 Variety =11.30, Soil ×Variety =25.28

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Table 8: Percent N2 (mg plant-1) fixed per plant of five uninoculated soybean varieties in five different soils

	Soil series					
Varieties	Goi	Agawtaw	Hake	Prampram	Simpa	Mean
Bengbie	75.9	76.8	86.5	80.2	84.3	80.7
TGx 1830-20E	84.8	79.4	85.7	85.6	81.7	83.4
TGx 1835-10E	83.7	81.1	85.8	83.6	77.8	82.4
Bragg	78.3	78.2	87.0	76.1	70.7	78.1
Mean	80.7	78.9	86.3	81.4	78.6	-

LSD ($P < 0.05$); Soil =4.3, Variety =4.3, Soil ×Variety =9.6

FIELD DATA JULY 2015