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N_2 fixation, carbon accumulation, and plant water relations in soybean (*Glycine max* L. Merrill) varieties sampled from farmers' fields in South Africa, measured using ^{15}N and ^{13}C natural abundance

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ABSTRACT

In this study, the natural isotopes of N (^{15}N) and C (^{13}C) were used to evaluate symbiotic N nutrition, C accumulation, and plant water-use efficiency in soybean varieties sampled from 37 farmers' fields across the soybean-producing region of South Africa. The data revealed significant differences in all the parameters measured. Shoot dry matter ranged from 17 to 104 g plant^{-1}, $\delta^{15}N$ from −1.11 to +5.51‰, percent N derived from fixation from 21 to 96%, and N-fixed varied from 18 to 298 kg N ha^{-1}. The high $\delta^{15}N$ and low %Ndfa in some soybean genotypes were due to inhibition of N_2 fixation by soil N uptake. Across the board, soybean variety PAN373 contributed the most symbiotic N (298, 242, and 217 kg N ha^{-1} in fields 3, 2 and 4, respectively, at Parys), followed by LS6164 (with 271 and 245 kg N ha^{-1} at Endcot field 1 and Devon field 2, respectively), and LS6150 (with 290 kg N ha^{-1} in field 1 at Parys). C concentration varied from 44 to 50% in soybean shoots, resulting in high shoot C ranging from 8 to 48 g C plant^{-1}. The $\delta^{13}C$ values of soybean shoots ranged from −27.3‰ to −21.1‰ in the 37 fields studied, with PAN1453 from Gransvlei field 1 and PAN737 from Parys field 4 exhibiting much greater $\delta^{13}C$ values (−21.1‰ and −23.1‰, respectively), and hence increased water-use efficiency. The positive correlation found between N-fixed and dry matter yield (r = 0.70***), N-fixed and C content (r = 0.62***), and N-fixed and C concentration (r = 0.35*) indicates a functional relationship between N_2 fixation and photosynthesis. Conventional tillage, as an agronomic tool, decreased water-use efficiency (or $\delta^{13}C$) in soybean plants possibly through alteration in soil structure and soil water retention. Due to a 2.6 °C higher daily maximum temperature in the North West Province than KwaZulu–Natal and Mpumalanga, soybean plants sampled from that province showed better growth, higher dry matter yield, and enhanced symbiotic performance. This argument was supported by a significantly positive correlation found between average daily maximum temperature and both dry matter yield (r = 0.39*), and N-fixed (r = 0.46**). Furthermore, the plants sampled from North West also showed much greater water-use efficiency due to a 127 and 67 mm less rainfall at that province than KwaZulu–Natal and Mpumalanga, respectively.

1. Introduction

Across Africa and South Africa in particular, soybean is in high demand for the livestock industry, as well as for use as food. With 40% protein and 20% oil in the grain, soybean (*Glycine max* L. Merrill) represents a very important source of dietary protein and oil for humans, and a high-protein feed for livestock production (Zarkadas et al., 2007). The soybean plant has the ability to form symbiosis with compatible soil bacterial strains of *Bradyrhizobium japonicum* (Jordan, 1982; Black et al., 2012), *Bradyrhizobium elkanii* (Kuykendall et al., 1992), *Bradyrhizobium liaoningense* (Xu et al., 1995) and *Sinorhizobium fredii* (Chen et al., 1988). Hungria et al. (2005) have argued that inoculating soybean genotypes with efficient strains of these bacteria in Brazil can allow the plant to meet its entire N needs from atmospheric N_2 fixation, and in so doing, contribute substantially to the N economy of cropping systems.

It has been estimated that about 50–60% of soybean N demand is met globally from biological N_2 fixation (Salvagiotti et al., 2008). However, the proportion of N derived from symbiotic fixation by this legume can vary hugely from 0 to 97% in some areas of North America (Keyser and Li, 1992). Where high numbers of effective bradyrhizobial strains are applied as inoculants, the North

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American soybean genotypes can contribute up to 311 kg N ha^{-1} under low soil N conditions (Keyser and Li, 1992). In Australia, on-farm studies have revealed 23–94% dependency on N_2 fixation for soybean's N nutrition (Peoples et al., 1995). In Africa, the soybean varieties that are currently promoted for farmer adoption include the TGx, or tropical glycine crosses (Abaidoo et al., 2007), which have the ability to effectively nodulate with indigenous soil rhizobia. These so-called promiscuous nodulating soybean genotypes can derive about 56–63% of their N nutrition from atmospheric N_2 fixation, and contribute between 79 and 122 kg N ha^{-1} under field conditions (Pule-Meulenberg et al., 2011). These levels of N_2 fixation in soybean have been reported to increase grain yield and seed protein, in addition to improving soil fertility (Fabre and Planchon, 2000).

However, one major factor affecting growth and N_2 fixation in soybean plants is soil water deficit. Even in the presence of adequate nutrient supply, water stress (drought) can reduce leaf photosynthesis and transpiration via closure of stomata (Merewitz et al., 2011), as well as alter gene expression, signalling pathways and cellular functioning in plants (Ribas-Carbo et al., 2005). About 40% yield reduction has been reported for soybean under conditions of soil water deficit (Manavalan et al., 2009). The ratio of dry matter produced per unit of water lost during photosynthesis (transpiration efficiency) is a critical measure of a plant's response to low soil moisture, and can be increased through either net photosynthesis, low transpiration, or both (Condon et al., 2004). Plant transpiration efficiency is strongly linked to the ratio of intercellular CO_2 and atmospheric CO_2 concentrations (i.e. C_i/C_a ratio), which is a function of stomatal conductance and leaf chloroplast demand for CO_2 during photosynthesis (Zhao et al., 2004). In general, plants tend to obtain more carbon (biomass) in exchange for leaf water transpired, thus improving transpiration efficiency. With crop species, however, a greater proportion of accumulated biomass is usually partitioned to the grain, the harvested product of photosynthesis.

During photosynthesis, atmospheric CO_2 is reduced to organic C by ribulose bisphosphate carboxylase-oxygenase (Rubisco). In the process, the Rubisco enzyme discriminates between $^{13}CO_2$ and $^{12}CO_2$ in C3 species (Farquhar et al., 1989). However, the level of discrimination against the heavier $^{13}CO_2$ in favour of the lighter $^{12}CO_2$ is related to the ratio of internal to external partial pressure of CO_2, which in turn is influenced by stomatal conductance and photosynthetic rates (Farquhar et al., 1989). The ^{13}C discrimination in C3 plants is therefore related to water-use efficiency, with higher ^{13}C enrichment being associated with greater water-use efficiency. Thus, plants developed under water stress tend to have high $\delta^{13}C$ values (or greater water-use efficiency), while those experiencing adequate soil moisture exhibit low $\delta^{13}C$, or reduced water-use efficiency (Ferrio et al., 2003). The ^{13}C isotope technique can therefore be used to study plant–water relations, and to select for water-use efficient crop species and genotypes in breeding programs (Condon et al., 2004; Blum, 2005). In nature, the instantaneous plant transpiration efficiency during photosynthesis and the long-term integrated water-use efficiency of plants are measured using gas-exchange studies and ^{13}C isotope analysis, respectively.

The aim of this study was to use the natural isotopes of N (^{15}N) and C (^{13}C) to evaluate whether symbiotic N nutrition and plant water relations in soybean varieties sampled from farmers' fields differ across the soybean-growing regions of South Africa. Additionally, we also measured photosynthetic C accumulation in seven farmers' fields using infra-red gas analyzer to ascertain if there were differences in C accumulation in soybean varieties grown in farmers' fields.

2. Materials and methods

2.1. Description of experimental sites

Field surveys were conducted from farmers' fields in three provinces of South Africa (i.e. KwaZulu–Natal, North West and Mpumalanga, which are the three major soybean growing provinces), and plants were sampled from a total of 37 farms (15 in KwaZulu-Natal, 12 in North West and 10 in Mpumalanga Province). The 37 farmers' fields surveyed were in the vicinity of 10 towns, namely Winterton and Bergville in KwaZulu–Natal, Devon, Eloff, Endicot, Leandra, and Nigel in Mpumalanga, Potchefstroom, Parys and Gransvlei in North West Province. By definition, farmers' fields represent farms designed, planted, and managed entirely by commercial soybean farmers for grain production without any input from researchers. In terms of cultural practice, prior to planting soybean seeds from all fields were inoculated with *B. japonicum* strain WB74, a commercial inoculant produced by Stimuplant Company in Pretoria, and Soygrow Company in Potchefstroom, South Africa. A monophosphate fertilizer (49% P at 220–300 kg P ha^{-1}) was applied by all farmers to their soybean crop at planting.

Annual rainfall data, as well as minimum and maximum monthly temperatures were obtained from the Agricultural Extension agents in each of the 10 towns used as study sites.

Table 1
Monthly maximum temperatures (°C) of study sites/towns where soybean plants were sampled.

Town	Temperature	2009			2010								
		Oct	Nov	Dec	Jan	Feb.	Mar	April	May	Jun	Jul	Aug	Sep
Bergville	Maximum	24	23	25	24	24	24	23	20	19	18	20	22
Winterton	Maximum	24	23	25	24	24	24	23	20	19	18	20	22
Potchefstroom	Maximum	27	28	26	28	27	26	24	22	18	19	22	24
Parys	Maximum	27	28	26	28	27	26	24	22	18	19	22	24
Devon	Maximum	27	26	27	26	26	25	23	20	18	18	21	25
Nigel	Maximum	27	27	26	26	26	25	23	20	18	18	21	25
Leandra	Maximum	25	25	26	25	24	24	21	19	16	16	20	22
Elloff	Maximum	27	26	28	27	26	24	23	21	19	18	21	24
Endcot	Maximum	25	27	27	26	26	25	23	20	18	18	21	25