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FIELD-6756; No. of Pages 11

ARTICLE IN PRESS

Field Crops Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

The response of wheat genotypes to inoculation with *Azospirillum brasilense* in the field

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ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form 18 July 2016
Accepted 18 July 2016
Available online xxx

Keywords:
Bacterial inoculation
Wheat yield
Genotype × bacterial strain interaction
Azospirillum brasilense.

ABSTRACT

The response of wheat (*Triticum aestivum*) genotypes to inoculation with different growth promoting bacterial inoculants is unclear. Five wheat genotypes that showed a differential response to inoculation with three *Azospirillum brasilense* strains under controlled conditions were sown in field experiments with and without inoculation in two environments in 2010 and 2011 in north-western New South Wales. Inoculum was applied in furrow to whole plots within a split plot design. Plant height, root architecture, above ground dry matter accumulation, relative chlorophyll, Normalized Difference Vegetation Index (NDVI), grain yield, biomass at maturity, tiller number and thousand kernel weight (TKW) were assessed. Inoculation improved some traits, particularly in the early growth phase; however these effects diminished with time. Little effect of bacterial inoculum on grain yield was observed with the exception of one genotype that produced significantly higher yield in the presence of inoculum. While genotype × inoculation interactions were significant for some traits including TKW, no interaction was observed for biomass and grain yield. The bacterial inoculum did persist in rhizospheric soil with time. However, persistence estimated throughout the season using the most probable number technique and terminal restriction fragment length polymorphism (T-RFLP), was observed to be higher early in the season in inoculated plots and reduced with time.

1. Introduction

Inoculation of *Azospirillum brasilense* on economically important crops has enhanced plant growth and productivity in many environments (Veresoglou and Menexes 2010). While much is known about *A. brasilense*, the effect of this plant growth promoting bacteria (PGPB) in the field is limited by factors that influence bacterial survival and activity in the rhizosphere.

In a large study, Zorita and Canigia (2009) evaluated 297 field experiments in Argentina between 2002 and 2006 and assessed the effect of inoculation with *A. brasilense* INTA Az-39 on wheat crop productivity. Positive responses were observed in 70% of the sites and the inoculated crop showed superior shoot and root vegetative growth with an average improvement in grain yield of 8%.

The attachment of bacteria to roots is an essential and necessary condition for the establishment of an effective association. However, this association is dependent upon the population density of active PGPB cells in the rhizosphere and their ability to compete with indigenous bacteria. Survival and persistence of inoculant bacteria in the rhizosphere, the effect of inoculum on the rhizosphere community, in particular the nitrogen fixing bacteria and the contribution of plant genotype to plant growth promotion by *Azospirillum* in the field have not been widely studied. Current evidence from field applications are not always consistent (Bowen and Rovira 1999; Zorita and Canigia, 2009). This inconsistency could be due to the failure of inoculant microorganisms to survive in competition with indigenous microorganisms in the soil and biotic and abiotic stresses may enhance this effect (Nelson 2004).

Monitoring plant growth promoting bacterial populations in the rhizosphere and their colonization and persistence after application is difficult because of the complexity of the indigenous bacterial community. Enumeration methods need to be specific to a particular species or strain and also determine viability. Culture dependent methods are ideal for determining viability but no single medium is suitable for isolating individual microbial strains from a complex community (Deaker et al., 2008). In addition, the method should provide consistent results under diverse conditions (Sutton, 2010). A technique for estimation of the most probable number (MPN) of *A. brasilense* based on nitrogen free semisolid media has been used previously (Baudoin et al., 2010). Another approach to

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http://dx.doi.org/10.1016/j.fcr.2016.07.012
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Please cite this article in press as: Kazi, N., et al., The response of wheat genotypes to inoculation with *Azospirillum brasilense* in the field. Field Crops Res. (2016), http://dx.doi.org/10.1016/j.fcr.2016.07.012

determine whether the inoculant strain has colonised and persists in the rhizosphere is to measure its effect on the microbial community using Terminal-Restriction Fragment Length Polymorphism (T-RFLP). T-RFLP is a PCR based DNA fingerprinting technique used for rapid analysis of the diversity of complex bacterial communities in different environments (Liu et al., 1997; Osborn et al., 2000) and it has been widely adopted for soil microbial studies. The T-RFLP method was recently used to analyze communities of bacteria, archaea, fungi, other phylogenetic groups or subgroups, as well as functional genes in different environments and the results were reported to be highly reproducible (Smalla et al., 2007; Sun and Liu 2013).

Various *Azospirillum brasilense* strains have been described including Sp7 (Tarrand et al., 1978) and its spontaneous mutant Sp7-S, first described by Katupitiya et al. (1995). These strains colonise wheat roots differently with Sp7 able to colonise the root surface and Sp7-S only the crevices where lateral roots emerge. The strain Sp245 produces IAA and was reported to have plant growth promoting effects on wheat (Spaepen et al., 2008). All three strains retained the capacity to fix N2, however this was not considered the major mechanism of plant growth promotion. *Azospirillum* also promotes plant growth through the production of phytohormone and Sp245 likely enhances plant growth more than Sp7 and Sp−7S because of increased IAA production. The nifH gene encodes the structural component of Fe nitrogenase (Poly et al., 2001) and can be differentiated from the often targeted 16S gene (Liu et al., 1997), representing all bacteria. Targeting the nifH gene might be expected to increase the sensitivity of screening procedures to community shifts as a result of inoculation.

Given the paucity of information on wheat genotype responses to inoculation with *Azospirillum* in the field, this study aimed to (i) determine the best combination of inoculant strains and wheat genotypes under varying levels of N stress in the field using different assessments of plant growth and grain yield and (ii) monitor the colonization and persistence of *Azospirillum* in the wheat rhizosphere in the field under dryland conditions in a sub-tropical wheat growing region. It was expected that colonization potential of the three strains would vary with different wheat root architectures and bacterial strain phytohormone production.

2. Materials and methods

2.1. Site description and crop rotation

Experiments were conducted during May-November in 2010 and 2011 at the IA Watson Grains Research Centre located near Narrabri in northwestern New South Wales (NSW) (30°19′0″ latitude and 149°46′0″ E longitude). The soil was a black vertosol with 2.7% organic matter, 3.7 N nitrogen weight/volume and pH 7.5 in the top 15 cm. The experiments sown in both years were conducted on different sites within the Narrabri research station but following the same 4-year crop rotation: barley; field pea; fallow; wheat. The site was managed using zero-tillage and weeds were chemically controlled as needed during the rotational period and during the winter season when experiments were sown.

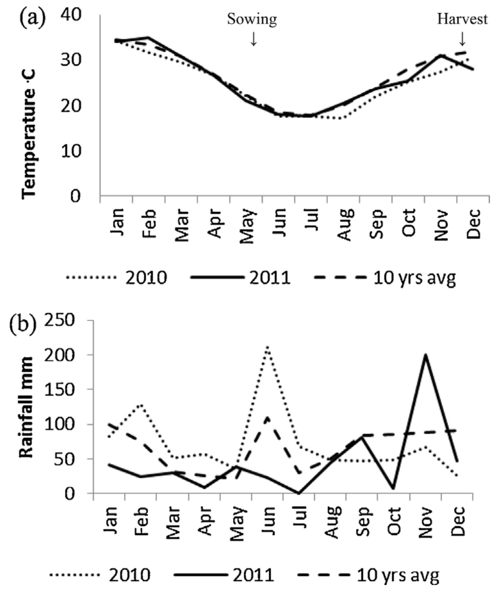

Fig. 1. Rainfall (a) and average maximum temperature (b) at Narrabri during 2010, 2011 and the 10 year average (source http://www.bom.gov.au/climate/data/).

2.2. Growing season description

Experiments were sown on May 20th in both years and harvested on November 20th 2010 and November 17th 2011. Irrigation was not applied in either year as rainfall was sufficient throughout the growth period. Narrabri is a summer dominant rainfall environment and experiments were sown on a full moisture profile. Fig. 1 shows the annual fluctuation in maximum temperature and rainfall at Narrabri in 2010 and 2011 and the 10-year average. The winter rainfall in both years was below average. However, the spring was much wetter than average and experiments received above average rainfall from boot stage until harvest. Spring maximum temperatures were considerably lower than the 10-year average in 2010 resulting in a longer and cooler booting, anthesis and grain-filling period. Temperatures in 2011 were slightly warmer but still below the long-term average from anthesis onwards.

2.3. Wheat genotypes evaluated

Five wheat genotypes were selected for the experiments in 2010 and 2011 (Table 1). These five genotypes were selected from a range of 23 diverse wheat genotypes which were grown in a controlled environment with bacterial treatments (data not presented). These were reduced using DNA fingerprints (based on Diversity Arrays Technology or DArT profiles) to ensure maximum genetic diversity and likelihood of responsiveness among the five selected genotypes. The five genotypes differed for various morphological characteristics including plant height and seed size. EGA GREGORY is a widely grown cultivar in the Narrabri region, SOKOLL and CROC_1/AE.SQUARROSA(205)//KAUZ/3/2*METSO are synthetic derivatives developed by the International Maize and Wheat Improvement Centre (CIMMYT) and therefore represent

Table 1
List of the genotypes evaluated in field trials in 2010 and 2011.

Pedigree	Agency & year	Description
1. EGA GREGORY	DEEDI (Department of Employment, Economic Development and Innovation)	Released Australian cultivar 2004
2. CBRD/KAUZ//KASO2	International Center for Maize and wheat Improvement	Advanced line
3. CROC_1/AE.SQUARROSA(205)//KAUZ/3/2*METSO	International Center for Maize and wheat Improvement	Synthetic derivative
4. SOKOLL	International Center for Maize and wheat Improvement	Synthetic derivative
1. 5 KRICHAUFF	University of Adelaide	Released Australian cultivar 1997

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