SUNTEXT REVIEWS

SunText Review of Biotechnology
ISSN: 2766-5097

Open Access
Research Article
Volume 5:1

Characterization, Selection and Evaluation of Temperature Tolerant Bradyrhizobium Japonicum Strains to Promote Soybean Cultivation in Tropical Soils Condition

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Received date: 01 September 2024; **Accepted date:** 09 September 2024; **Published date:** 17 September 2024

Citation: Prasad K (2024) Characterization, Selection and Evaluation of Temperature Tolerant Bradyrhizobium Japonicum Strains to Promote Soybean Cultivation in Tropical Soils Condition. SunText Rev Biotechnol. 5(1): 151.

DOI: https://doi.org/10.51737/2766-5097.2024.051

Abstract

One Kind Of Legume-Root Nodulating, Microsymbiotic Nitrogen-Fixing Bacterium Is Called Bradyrhizobium Japonicum. The Bacterial Species Is One Of Many Rod-Shaped, Gram-Negative Bacteria Known As Diazotrophic Bacteria; These Bacteria Fix Nitrogen After Settling Inside The Legume (Fabaceae) Family's Root Nodules. The Current Experiment Was Conducted To Collect Data On In Vitro Physiological Temperature Tolerance Of Sixteen Bradyrhizobial Strains Isolated From Soybean Root Nodules Collected From Various Soybean Growing Fields Of Bundi And Udaipur Regions Of Rajasthan, India. The Extent Of Endurance Of Bradyrhizobial Strains To Various Temperatures Was Investigated By Incubating The Bacterial Culture At Different Temperatures Ranging From 250c - 550c. The Bradyrhizobial Growth In 20e Broth Was Observed As Od540nm And Interpreted In Terms Of The Temperature Tolerance Of The Strains. Results Indicated A Decline In Bacterial Growth With The Elevated Temperature. At 400c, Five Bradyrhizobial Strains Namely Bj335-1, Bj335-2, Bpk-1, Uj335-3 And Uj335-4 Showed A 5.09% To 19.45% Decline In Od540nm Compared To The OD Values Recorded At 250c And Rest Of The Strains Showed 22.54% To 44.54% Decrease. At 500c Only Two Strains Namely Bpk-3 And Uj335-1 Could Exhibit Noticeable Growth In The 20e Broth While The Rest Of The Bradyrhizobial Collection Exhibited Insignificant Growth And None Of The Strains Was Able To Tolerate The Temperature Beyond 550c. However, The Uj335-1 Strain Was Still Tolerant At 550c Temperature Showing Noticeable Turbidity In The 20e Broth. The Present Study On The Temperature Response Of Bradyrhizobia Could Be Useful In Identifying Better Strains Suited To Soils Having Elevated Temperature; A Uniform Feature As Also Exists In The Soybean Cultivation Region Of Rajasthan, Indian.

Keywords: Evaluation; Soybean; Temperature; Tropical soil; Bradyrhizobium japonicum

Introduction

Microorganisms, particularly microscopic organisms, increase soil richness through supplement reusing like carbon, nitrogen, sulfur, phosphorus, iron and potassium. Microorganisms deteriorate dead natural matter and deliver basic mixtures in the dirt, which can be taken up by plants. Nitrogen-fixing bacteria (NFB) fix atmospheric nitrogen and increase the nitrogen content in the soil, which can be readily absorbed by plants and improve growth and productivity. They likewise further develop soil construction and increase the water-holding capacity of the soil. A kind of soil bacteria known as rhizobia causes root nodules in legume roots by infecting the roots. Rhizobia are soil-dwelling microorganisms that, upon infection, cause legumes to develop nodules where they fix atmospheric nitrogen gas (N_2) and convert it into a form of nitrogen that is more easily used. The legume uses the nitrogen that is exported from the nodules to thrive. After the legume dies, the disintegration of the nodule releases the rhizobia back into the soil, where it can either survive on its own or infect a new legume host. One sort of legume-root nodulating, microsymbiotic NFB is called

Bradyrhizobium japonicum. B. japonicum is coated with legume seed to increase root development and improve crop yields, particularly in areas where the bacterium is not native. Often the inoculation is adhered to the seeds before planting using a jaggery/gum solution. They are a widespread type of soil-dwelling bacteria that may work in symbiotic partnerships with types of leguminous plants where they fix nitrogen in exchange for carbohydrates from the plant. Like other rhizobia, they could fix atmospheric nitrogen into forms readily available for other organisms to use. Since 1957, a strain of B. japonicum has been utilized as a model organism. It is widely used to study molecular genetics, plant physiology, and plant ecology due to its relatively superior symbiotic nitrogen-fixation activity with soybeans compared to other rhizobia species. The primary goal of agricultural microbiology is to comprehensively explore the interactions between beneficial microbes (BMs) such as bacteria and fungi with crops. It also deals with the microbiology of soil fertility (SF), such as microbial degradation of organic matter (OC) and soil nutrient transformations (SNT). Healthy populations of beneficial bacteria/fungi can help suppress pathogens and pests, promote plant growth (PG), and increase quality yield. The growth and activity of the N2 fixing plants are restricted by several environmental conditions. The process of fixing N2 in the Rhizobium-legume symbiosis is strongly correlated with the host plant's physiological state. Within the soil, rhizobia frequently encounters various stresses that affect their growth, the initial steps of symbiosis, and the efficiency of nitrogen fixation [1-3]. Among several environmental conditions, which are limiting factors, water stress, salinity, temperature extremes and pH stress are the most problematic. A competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation (NF) as these limiting factors impose limitations on the vigour of the host legume [4,5]. Since rhizobial populations vary in their tolerance to major environmental factors, screening for tolerant strains has been pursued by several researchers [2,6-17]. One of the main obstacles to BNF in legume crops is the high soil temperature found in tropical areas. Temperatures in these regions average above 400C may affect symbiotic relationships, nitrogen content and plant production [18,19]. Therefore, temperature tolerance findings of rhizobia are imperative to selecting heat-tolerant strains for tropical conditions. Michaels [20]. found heat-inducible proteins in heat-sensitive and heat-tolerant strains and demonstrated acquired thermotolerance in the heat-tolerant strain Rhizobium tropici CIAT899. Bayoumi [21]. additionally explored the impacts of various abiotic factors (acridity, saltiness, nitrate, and temperature) on the development pace of root-knob microbes (Rhizobium and Bradyrhizobium) strains in vitro and announced that strains separated from Vicia faba L., Coronilla varia L. furthermore, Lupinus albus L. shown a huge variety in resilience of the previously mentioned factors. Pinto [22]. Evaluated the effects of heat on growth, survival, symbiotic performance, and genomic modifications in effective R. tropici and R. leguminosarum bv. phaseoli strains isolated from Cerrado soils and suggested that the strategies used in their study to evaluate survival capacity, N2 fixation performance and genetic stability after thermal stress could be useful in selecting efficient and stable Rhizobium strains to be used as inoculum for bean plant cultivation in tropical soil conditions. Given the above discussion, further work is still needed to increase our knowledge of the rhizobial ecology under suboptimal environmental conditions such as elevated temperature which may constitute a strategy for improving legume-rhizobium symbiosis in adverse environments of various soybean growing regions of Rajasthan, India. With this aim in mind, a current study was conducted, and data were collected on the in vitro physiological temperature tolerance of sixteen authenticated bradyrhizobial strains.

Materials and Methods

Isolation, characterization, authentication, and nomenclature of Bradyrhizobial strains

Isolate of B. japonicum strains were obtained from root nodules of field-grown soybean plants (cv. PK 472) collected from Adaptive Trial Centre (ATC), Bundi, Rajasthan, India (Figure 1). The serial dilution agar plate method as described in Somasegaran and Hoben [23] was employed with 20E medium [24] for Bradyrhizobium strain isolation. Isolates were streak and purified on the same medium and the purified isolates were subjected to an authentication test in growth pouches (procured from Mega International, USA) under controlled environmental conditions (day length, 14 h; temperature, 28±1°C, light intensity, 12000 lux; humidity, 70-80%). Sixteen authenticated strains were maintained as frozen glycerol stocks at - 40°C. These strains were nomenclature as their first letter depicting the region of origin (B-Bundi; U- Udaipur) and a numeric figure given at the end showing the specific strain assigned number. J335 and PK represent the soybean cultivars JS 335 and PK 472 respectively (Table 1).

Temperature tolerance of bradyrhizobial strains

Bradyrhizobial culture suspensions were inoculated in tubes containing 20E broth. These tubes were incubated at various temperatures ranging from 25 ^{0}C to 55 ^{0}C. The bacterial growth at various temperatures was measured by recording optical density at 540 nm using a Systronics model 106 spectrophotometer (Table 1). In this experiment, 28 ±1^{0}C was taken as ‘control’ or ‘reference’ temperature of thermal exposure.

Results and Discussion

The extent of tolerance of bradyrhizobial strains to various temperatures was studied by incubating the bacterial culture at different temperatures ranging from 25^{0}C to 55^{0}C. Comparative growth of the sixteen bradyrhizobial strains in terms of OD540nm at 55^{0}C temperature has been shown in Figure 3. It is evident from Table 1 and Figure 2 that there was a decline in bacterial growth with the elevated temperature. At 25^{0}C maximum growth (0.945) was shown by UJ335-5 while minimum growth (0.589) was shown by BPK-1 and BPK-6. At 28^{0}C and 30^{0}C luxuriant growth ranging from 0.874 -1.304 OD540nm and 0.845-1.187 OD540nm respectively was recorded for the studied bradyrhizobial strains. At 35^{0}C a gradual decrease in OD540nm compared to 30^{0}C was noticed and the maximum OD540nm (0.989) at this temperature was recorded for UJ335-1 while minimum OD540nm (0.675) was observed for BPK-6.

Figure 1: *Location map of Soybean cultivation area at Bundi, Rajasthan, India.*

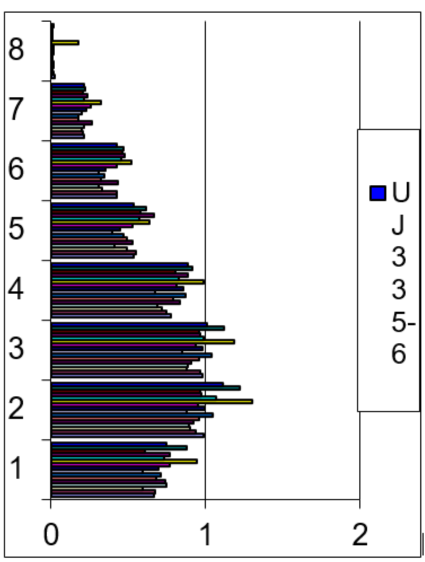

Figure 2: *Temperature tolerance of sixteen bradyrhizobial strains (columns showing OD540nm values; 1-25^{0}C, 2-28^{0}C, 3-30^{0}C, 4-35^{0}C, 5-40^{0}C, 6-45^{0}C, 7-50^{0}C, 8-55^{0}C).*

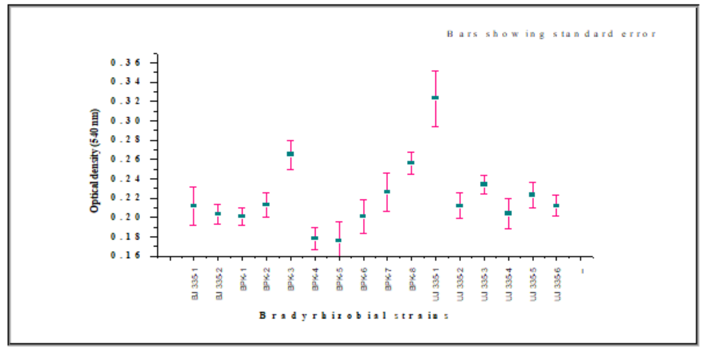

Figure 3: *Comparison of sixteen bradyrhizobial strains with regard to high temperature (55^{0}C) tolerance.*

At 40^{0}C, five bradyrhizobial strains namely BJ335-1, BJ335-2, BPK-1, UJ335-3 and UJ335-4 showed a 5.09% to 19.45% decline in OD540nm compared to the OD values recorded at 25^{0}C and rest of the strains showed 22.54% to 44.54% decrease. At 45^{0}C OD540nm ranged from 0.306 – 0.521. At 50^{0}C, this decrease in OD values was more obvious where the OD values ranged from 0.176 – 0.323 and only two strains namely BPK-3 and UJ335-1 could exhibit noticeable growth (0.265 and 0.323 respectively) in the 20E broth. However, at 55^{0}C temperature a drastic decrease in terms of insignificant OD540nm was encountered which ranged from 0.006 – 0.021 for fifteen bradyrhizobial strains. However, UJ335-1 strain was still tolerant at this temperature showing noticeable turbidity (0.178 OD540nm) in the 20E broth (Figures 2,3). Several studies have reported that rhizobial growth is adversely affected by high soil temperature [14,15] [26-28]. Consequently, temperature tolerance studies of sixteen bradyrhizobial strains were conducted. Parallel to these studies, in the present investigation, a decline in bradyrhizobial growth with increased temperature was also realized. The optimum temperature range for bradyrhizobial growth in the current study was 28^{0}C-30^{0}C. A few past Scientists likewise affirmed this finding by detailing that the ideal temperature for the development of root nodulating bacteria ranged from 25°C-30°C [29,30]. However, the inhibitory effect of elevated temperature (45°C) was visible on the growth response of bradyrhizobia since moderate growth was recorded for majority of the strains. This result corroborates the previous findings of Werner [31] (1992) who stated that survival of the majority of Bradyrhizobium strains is strongly reduced above 40oC. Moreover, LaFavre and Eaglesham also reported that the nodulation of soybean was markedly inhibited at 42°C and 45°C for 12 hours and 9 hours days. UJ335-1 was found to be the highest temperature-tolerant strain in the current study, which showed considerable growth even at a temperature as high as 55^{0}C. However, no strain could survive at temperatures beyond 55^{0}C. On the other hand, Mahobia and Mahna [13] while characterizing Prosopis rhizobia reported that all the strains could tolerate temperatures up to 55^{0}C.

Table 1: *Effect of temperature on growth of sixteen bradyrhizobial strains of soybean*.

Brady-rhizobial strains	Range of temperature							
	25ºC	**28ºC**	**30ºC**	**35ºC**	**40ºC**	**45ºC**	**50ºC**	**55 ºC**
BJ 335-1	0.663±0.023	0.985±0.022	0.978±0.034	0.773±0.047	0.534±0.032	0.427±0.023	0.212±0.020	0.021±0.003
BJ 335-2	0.673±0.034	0.937±0.041	0.968±0.043	0.749±0.011	0.545±0.017	0.428±0.030	0.203±0.017	0.016±0.002
BPK-1	0.589±0.034	0.896±0.045	0.879±0.032	0.713±0.034	0.489±0.029	0.328±0.047	0.201±0.019	0.011±0.001
BPK-2	0.743±0.045	0.892±0.015	0.886±0.012	0.689±0.008	0.412±0.050	0.310±0.012	0.213±0.023	0.017±0.003
BPK-3	0.739±0.046	0.923±0.066	0.905±0.049	0.836±0.048	0.525±0.043	0.435±0.042	0.265±0.015	0.019±0.002
BPK-4	0.678±0.076	0.957±0.026	0.958±0.021	0.786±0.087	0.489±0.065	0.321±0.041	0.178±0.011	0.008±0.001
BPK-5	0.708±0.057	1.048±0.048	1.036±0.050	0.867±0.027	0.467±0.034	0.343±0.022	0.176±0.019	0.006±0.001
BPK-6	0.589±0.011	0.874±0.039	0.845±0.093	0.675±0.048	0.398±0.029	0.306±0.012	0.201±0.027	0.014±0.002
BPK-7	0.698±0.038	0.993±0.043	0.978±0.051	0.853±0.077	0.447±0.038	0.355±0.017	0.226±0.028	0.017±0.003
BPK-8	0.766±0.059	0.948±0.048	0.939±0.017	0.812±0.094	0.527±0.041	0.426±0.038	0.256±0.021	0.019±0.002
UJ 335-1	0.945±0.049	1.304±0.023	1.187±0.018	0.989±0.083	0.637±0.055	0.521±0.048	0.323±0.029	0.178±0.014
UJ 335-2	0.732±0.044	1.065±0.033	0.984±0.051	0.824±0.043	0.567±0.034	0.455±0.038	0.212±0.018	0.006±0.000
UJ 335-3	0.767±0.050	0.973±0.019	0.964±0.073	0.882±0.098	0.668±0.056	0.479±0.038	0.234±0.010	0.008±0.001
UJ 335-4	0.609±0.037	0.968±0.036	0.958±0.082	0.806±0.099	0.578±0.066	0.463±0.043	0.204±0.016	0.010±0.001
UJ 335-5	0.879±0.044	1.217±0.067	1.117±0.043	0.911±0.048	0.611±0.040	0.465±0.014	0.223±0.017	0.011±0.002
UJ 335-6	0.749±0.012	1.113±0.039	1.006±0.088	0.887±0.101	0.534±0.027	0.421±0.023	0.212±0.011	0.016±0.003
*Data indicate mean value of three replicates: ± standard error.								

Conclusion

Overall evaluation of the data showed that the growth of bradyrhizobia decreased with increasing temperature. All the investigated bradyrhizobia strains grew abundantly at temperatures between 28°C and 30°C. At 40°C, at least nine bradyrhizobia strains showed a significant decrease in OD values ranging from 27.87% to 44.54% compared to the OD values at 25°C. At 50°C, only two strains, BPK-3 and UJ335-1, were able to show significant growth in 20E broth, while the rest of the bradyrhizobia collection showed only slight growth and none of the strains could tolerate temperatures above 55°C. These temperature-tolerant strains can also be selected to evaluate the possibility of symbiosis under elevated temperature in field conditions, a common trait found in various soybean-growing regions of Rajasthan, India.

Funding

The author declares no financial support for the research, authorship, or publication of this article.

Author contributions

The author declare that the author contributed, approve of this work, and take full responsibility.

Conflict of interest

The author declares no conflict of interest.

Data availability statement

Data supporting these findings are available within the article.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

The author declare that no human participants were involved in this study. Sample availability the author declare that no physical samples were used in this study.

References

1. Zahran HH. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol Biol Rev. 1999; 63: 968-989.
2. Prasad K. Symbiotic Endophytes of Glomalin am fungi, rhizobium, and PGPR potential bio stimulants to intensive global food production for sustainable agriculture system. J Microbes Res. 2023; 2: 1-23.
3. Prasad K, Rajasekaran S, Pandey, RK, Rajat K. Multiple functions of microbes to improve food and soil nutrition in support of environment management to maintain global agricultural practices. J Energy Environ Sci. 2024; 2:000114.

4. Brockwell J, Bottomley PJ, Thies JE. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. 1995; 174: 143-180.
5. Thies JE, Woomer PL, Singleton PW. Enrichment of Bradyrhizobium spp. Population in soil due to copping of the homologous host legume. Soil Biol Biochem. 1995; 27: 633-636.
6. Keyser HH, Somasegaran P, Bohlool BB. Rhizobial ecology and technology, In Meeting, F. B. (ed.), Soil microbial ecology: applications in agricultural and environmental management. Marcel Dekker, Inc., New York. pp. 1993; 205-226.
7. Andres JA, Correa NS, Rosas SB. Survival and symbiotic properties of Bradyrhizobium japonicum in the presence of thiram: isolation of fungicide resistant strains. Biol Fertil Soils. 26: 141-145.
8. Baraibar A, Frioni L, Guedes ME, Ljunggren H. Symbiotic effectiveness and Ecological characterization of indigenous Rhizobium loti populations in Uruguay. Pesq. Agropec Bras. 1999; 34: 1011-1017.
9. Kumar H, Arora NK, Kumar V, Maheshwari DK. Isolation, characterization and selection of salt tolerant rhizobia nodulating acacia catechu acacia nilotica. 1999; 26: 279-288.
10. Ulrich A, Zaspel I. phylogenic diversity of rhizobial strains nodulating robinia pseudoacacia l. microbiol. 2000; 146: 2997-3005.
11. Arora NK, Kumar V, Maheshwari DK. Isolation of both fast and slow growing rhizobia effectively nodulating a medicinal legume, mucuna pruriens. 2000; 29: 121-137.
12. Saadallah K, Drevon JJ, Hajji M, Abdelly C. Genotypic variability for tolerance to salinity of n_2-fixing common bean (phaseolus vulgaris). 2001; 21: 675-682.
13. Mahobia V, Mahna SK. Characterization of rhizobia isolated from prosopis cineraria in Jodhpur region, Rajasthan, India. 2002; 5: 3-5.
14. Sridhar KR, Arun AB, Narula N, Deubel A, Merbech W. Patterns of sole-carbon-utilization by fast growing coastal sand dune rhizobia of the southwest coast of India. Eng Life Sci. 2005; 5: 425-430.
15. Meghvansi MK, Prasad K, Harwani D, Mahna SK. Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and Bradyrhizobium japonicum in the alluvial soil. Euro J Soil Biology. 2008; 44: 316- 323.
16. Prasad K. Stimulation impact of rhizosphere microbe's glomeromycota am fungi and plant growth promoting rhizobacteria on growth, productivity, lycopene, β-carotene, antioxidant activity and mineral contents of tomato beneath field condition cultivated in western Ghats covering semi-arid region of Maharashtra, India. J Bioscie Biomedic Eng. 2021; 2:1-14.
17. Prasad K. Stimulation effect of rhizospheric microbes' plant growth promoting rhizobacteria. Glycoprotein producing am fungi and synthetic fertilizers application on growth, yield, nutrient's acquisition and alliin content of garlic cultivation under field conditions in southeast region of Rajasthan, India. Advan Earth Envi Sci. 2022; 3:1-15.
18. Dudeja SS, Khurana AL. Persistence of bradyrhizobium sp. (cajanus) in sandy loam soil. Soil Biol Biochem. 1989; 21: 709-713.
19. Hungria M, Franco AA, Sprent JI. New sources of high temperature tolerant rhizobia for phaseolus vulgaris l. plant soil. 1993; 149: 103-109.
20. Michiels J, Verreth C, Vanderleyden J. Effects of temperature stress on bean-nodulating rhizobium strains. Appl. Environ Microbiol. 1994; 60: 1206-1212.
21. Pinto PP, Raposeiras R, Macedo AM, Seldin L, Paiva E, Sa NMH. Effects of high temperature on survival, symbiotic performance and genomic modifications of bean nodulating rhizobium strains. Rev Microbiol. 1998; 29: 295-300.
22. Somasegaran P, Hoben HJ. Handbook for rhizobia: methods in legume-rhizobium technology. Springer, New York. 1994.
23. Prasad K. Effect of dual inoculation of arbuscular mycorrhiza fungus and cultivar specific bradyrhizobium japonnicum on the growth, yield, chlorophyll, nitrogen and phosphorus contents of soybean (glycine max (l.) merrill.) grown on alluvial soil. J Innova Appl Rese. 2021; 4:1-12.
24. Werner D, Wilcockson J, Zimmermann E. Adsorption and selection of rhizobia by ion exchange papers. Arch Microbiol. 1975; 105: 27-32.
25. Osa-Afiana LO, Alexander M. Differences soil among cowpea rhizobia in tolerance to high temperature and desiccation in. Appl. Environ Microbiol. 1982; 43: 435-439.
26. Kluson RA, Kenworthy WJ, Weber DF. Soil temperature effects competitiveness and growth of Rhizobium japonicum induced chlorosis of soybean. Plant Soil. 1986; 95: 201-207.
27. Kennedy AC, Wollum AG. Enumeration of bradyrhizobium japonicum subjected to high temperature- comparison of plate count, most probable number and fluorescent antibody techniques. Soil Biol Biochem. 1988; 20: 933-938.
28. Gaur, YD. Microbiology, physiology and agronomy of nitrogen fixation.legume –rhizobium symbiosis. In Proc. Indian Natl Sci Acad. 1993; 59: 333-358.
29. Harwani D. Biodiversity and efficiency of bradyrhizobial strains and arbuscular mycorrhizal fungi of soybean cultivars grown in haroti region of Rajasthan. Ph. D. Thesis, Maharshi Dayanand Saraswati University, Ajmer, India. 2006.
30. Werner D. The Brady rhizobium/rhizobium fabales symbiosis. In Werner, d. symbiosis of plants and microbes. Chapman and hall, London. 1992; 49-151.
31. Lafavre AK, Eaglesham ARJ. Effects of high soil temperature and starter nitrogen on the growth and nodulation of soybeans. Crop Sci. 1987; 27: 742-745.