Original Research Article  

Genetic Variability for Nitrogen Use Efficiency in Interspecific and Intergeneric Hybrids of Sugarcane

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Abstract

Nitrogen is one of the most important mineral nutrients required for plant development especially for tillering and vegetative growth. Management of nitrogenous fertilizers poses a significant challenge in sugarcane cropping system as the efficiency of utilization of nitrogen is very poor. Improving the Nitrogen Use Efficiency (NUE) is imperative to achieve the maximum cane yield with less N inputs. In this study, 32 diverse sugarcane pre-breeding genetic stocks were evaluated with two levels of nitrogen (N₀ and N₁₀₀) for agronomic, juice quality, biomass traits and Agronomic Nitrogen Use Efficiency (AgNUE). Significant genetic variability was observed among levels of nitrogen and genotypes. Wider differences were observed between phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) indicating the role of nitrogen levels (N₀ and N₁₀₀) in trait expression. Maximum agronomic efficiency was observed for interspecific hybrids of Saccharum spontaneum (77.92 kg of dry biomass/kg of nitrogen) followed by intergeneric hybrid derivatives of Erianthus procerus (52.61 kg of dry biomass/kg of nitrogen). The study also revealed the early generation hybrids of S. spontaneum and E. procerus recorded maximum AgNUE could be the potential sources for developing nitrogen efficient varieties in sugarcane. Therefore, these genotypes further considered for utilization in crop improvement programmes for development of elite breeding pools for nitrogen use efficiency.

Keywords
Sugarcane, Agronomic nitrogen use efficiency (AgNUE)

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Introduction

Sugarcane is an important C₄ crop cultivated in the tropics and subtropics for production of both sugar and bioenergy (Waclawovsky et al., 2010; Dias et al., 2011; Smithers, 2014; Leal et al., 2016). Globally, sugarcane is cultivated in an area of 26.52 mha with a production of 1877.10 million tonnes of sugarcane and 172.36 million tonnes of sugar production during 2017-18. It is one of the most promising agricultural biomass sources and nearly one-third of biomass (leaves and tops) is suitable for sustainable energy production and economically viable for generating bioelectricity (Smithers, 2014). Nitrogen is the most limiting crop nutrition which is essential for getting desired crop
yields. Excessive use of nitrogen and cultivation of nitrogen inefficient cultivars promotes global climatic changes as emission of nitrous oxide is evident from the soil (Sornpoon et al., 2013 and Carmo et al., 2013). Sugarcane being a high biomass producing crop, requires larger quantities of fertilizer nitrogen with split doses during various stages of crop growth. The amount of nitrogen applied to sugarcane varies with different agroclimatic regions and cultivation practices followed. In India, it varies between 150kgs/ha in subtropical to 300 kg/ha in tropical India with additional 25% of N in ratoon crops. However, more than 50 per cent of Nitrogen supplied to the crop is lost through evaporation (Prammanee et al., 1988 and Prasertsak, 2002).

It clearly shows that there is a need to develop nitrogen efficient sugarcane genotypes which not only contributes significantly for sustainable sugarcane production but also reduces cost of sugarcane cultivation. The genetic variability available for Nitrogen Use Efficiency (NUE) in the parental gene pool is a pre requisite for designing appropriate breeding strategies (Garnett et al., 2015). Evaluation of large diverse population for NUE revealed the existence of significant genetic variation for N utilization in many crops (Rice, Singh et al., 1998; Barley, Sinebo et al., 2004;wheat, Le Gouis et al., 2000; Sugarcane, Robinson et al., 2007)which could serve as an initial donors for different NUE traits. However, several authors reported that due to modern plan breeding, selection under optimal N would have eroded the useful variation especially below ground root traits (wheat, Siddique et al., 1990;lettuce, Johnson et al., 2000). In a review on genetic variation for NUE in modern wheat, Hawkesford (2017) concluded that limited variation in the modern cultivars and suggested for broader germplasm screening for major improvement for N uptake and utilization. There are only few published report on NUE in sugarcane and it is not known the extent of NUE in diverse base materials of different interspecific and intergeneric hybrids. In view of this, the present experiment was conducted with following objectives i) to find out the species/genera related to sugarcane contributes to NUE ii) to identify pre-breeding genetic stocks with high NUE to further utilize in the sugarcane breeding programme for developing cultivars with high NUE.

Materials and Methods

Genotypes, nitrogen rates, and experimental design

Thirty two sugarcane pre-breeding genetic stocks with broader genetic base derived from different interspecific and intergeneric hybrids were selected for the study. The list of clones along with its parentage are given in the table 1. The selected clones were a part of pre breeding programme at ICAR-Sugarcane Breeding Institute, Coimbatore, India.

The trial was conducted at East Chithirai Chavadi farm of ICAR-Sugarcane breeding Institute, Coimbatore, Tamil Nadu, India (110 N,770E, 427MSL altitude) during 2014-16. The soil analysis was done by sampling at 45cm depth. The soil is a clay loam soil with a PH of 7.7, EC of 0.38 ds m-1 and organic carbon of 0.55. The soil is medium in N with 228.57 kg ha-1 and low in P (27.30 kg ha-1) and high in K (718.06 kg ha-1).

The clones were planted in two rows of 6m length with an inter row spacing of 0.9m. All the recommended agronomic practices except nitrogen fertilization were followed to raise good crop. Phosphorus was applied as basal and Nitrogen and Potassium were applied in two splits i.e 45 and 90DAP. Atrazine was applied as a pre emergence herbicide at 3 DAP to control broad leaved weeds. The
experiment was laid out in a split plot design with two levels of Nitrogen (no Nitrogen designated as N0 and recommended dose of Nitrogen (280kgha\(^{-1}\)) designated as N100.

**Data collection**

The following data viz., number of tillers (‘000/ha at 120 days), stalk height at harvest (360 days), number of stalks (‘000/ha at 360 days), stalk diameter (cm) and single stalk weight (kg) at harvest, fresh and dry biomass yield (tha\(^{-1}\)) were recorded in each plot and in each replication. Five stalks were cut and crushed in a crusher and the extracted juice was used to estimate juice quality parameters viz., juice brix%, sucrose %, commercial cane sugar %, purity %, extraction %). The fibre content(%) was estimated as detailed in Mohanraj and Nair (2014). Agronomic nitrogen use efficiency (AgNUE) defined as ‘cultivar produces large quantity of harvestable biomass per unit of nitrogen supplied’ was estimated as described by Good et al., (2004).

\(\text{AgNUE} \)  

Dry biomass under – Dry biomass under  
N 100 N0  

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Quantity of Nitrogen applied

**Statistical analysis**

Statistical analysis was performed for split plot design as described in Gomez and Gomez (1984) and total variability partitioned into variability due to nitrogen, genotypes and their interaction effects. The total variability due to genotypes further portioned into five categories (Kempthorne, 1957) viz., variability due to commercial clones (Co canes), interspecific hybrids of \(S. \) spontaneum (ISH) and intergeneric hybrid derivatives with \(E. \) arundinaceus (IGEA),backcross derivatives of \(E. \) procerus (IGEP) and intergeneric hybrid derivatives of sugarcane and sorghum (IGSS). Genotypic and phenotypic coefficient of variance, heritability (broad sense), and genetic advance as percent mean (GA) were estimated as described by Burton and deVane (1953) and Johnson et al., (1955).

**Results and Discussion**

**Analysis of variance and genetic variability parameters**

Analysis of variance revealed the presence of significant variation between two levels of nitrogen (N0 and N100) and among genotypes for all the traits studied. Further, partitioning of total genetic variability into mean sum of square due to Co canes and ISH had shown significant genetic variation for all the traits except no. of tillers. IGEP had shown significant genetic variation for most of the traits except for stalk diameter and single stalk weight whereas IGSS recorded significant mean sum of squares only for quality related traits viz., sucrose (%), purity (%), CCS (%) and fibre content (%).

Genetic variability parameters such as phenotypic coefficient of variability (PCV), genotypic coefficient of variability(GCV), heritability (h\(^2\)) and genetic advance (GA) as per cent mean for N0 and N100 is presented in table 2.Higher range, GCV and PCV for most of agronomic and biomass attributing traits was recorded in N100 and for juice quality parameters in N0. Wider differences were observed between GCV and PCV indicating the role of nitrogen levels (N0 and N100) in trait expression. Stalk diameter (cm), single stalk weight (kgs), number of stalks, juice brix %, sucrose %, purity %, CCS%, juice extraction %, fibre content (%) showed relatively high heritability and stalk height (cm) showed relatively moderate heritability under both levels of nitrogen. However, tiller number, fresh and dry biomass yield (tha\(^{-1}\))
showed relatively higher heritability of 0.71, 0.69, 0.72 under N\textsubscript{100} and low heritability of 0.21, 0.31 and 0.36 under N\textsubscript{0} respectively.

**Performance of agronomic traits at N\textsubscript{0} and N\textsubscript{100} levels**

Agronomic traits viz., no. of tillers, no. of stalks, stalk diameter, single stalk weight, juice quality traits such as juice extraction (%) and biomass attributing traits viz., fresh biomass yield and dry biomass yield recorded significantly high yield in N\textsubscript{100}. Juice quality parameters such as brix %, sucrose %, juice purity %, CCS (%), fibre(%) and dry matter (%) recorded significantly higher mean under N\textsubscript{0}.

AgNUE was estimated to assess the genotypes for accumulation of biomass per unit of nitrogen application. The mean AgNUE was 41.24 kg of dry biomass per kg of nitrogen supplied.

Maximum agronomic efficiency was observed for ISH (77.92 kg of dry biomass/kg of nitrogen) followed by IGEP (52.61 kg of dry biomass/kg of nitrogen), IGSS (40.18 kg of dry biomass/kg of nitrogen), IGEA (30.88 kg of dry biomass/kg of nitrogen) and the lowest agronomic efficiency was observed in Co canes (23.09 kg of dry biomass/kg of nitrogen) (Table 3).

Sugarcane is high biomass producing crop used for both generation of sugar and bioenergy and other industrial use products through usage of bagasse and trash (Dias et al., 2011; Furlan et al., 2013; Smithers, 2014; Leal et al., 2016). It is a high nitrogen demanding crop and efficient nitrogen management could able to improve sustainable sugarcane production. Excessive applications of nitrogen negatively impact the environment in sugarcane (Thorburn et al., 2017) and emission of greenhouse gases such as nitrous oxide (N\textsubscript{2}O) is evident from sugarcane fields (Signor et al., 2013; Sornpoon et al., 2013; Carmo et al., 2013). Excessive and indiscriminate usage of nitrogen causes global climatic change and emission due to agriculture is the third largest contributor to global greenhouse gases (Gelbert, 2012).

Global warming induced carbon fertilization with improved nitrogen use efficiency projected to increase in 24–31% increase in global agricultural N\textsubscript{2}O emissions by 2040–2050 (Kanter, 2016). Heavy fertilizer loads contaminates the aquatic environments like lakes, rivers, oceans with water soluble nitrates causes ecological disorders like dead zones and damages aquatic life diversity (Good et al., 2004). Contamination of drinking water with nitrogen causes methemoglobinemia or blue baby syndrome (Knobeloch et al., 2000).

Therefore, careful and effective crop management through agronomic practices and deployment of nitrogen use efficient genotypes could able to minimize the negative impact on environment, and development of nitrogen use efficient sugarcane cultivars through breeding, variety selection and genetic modification has gained the greater importance(Wood et al., 2010).

Set of thirty two sugarcane clones consisting of commercial canes and prebred genetic stocks derived from interspecific and intergeneric hybrids showed significant mean sum of squares and relatively higher values of range, GCV, PCV for most of the traits under both N\textsubscript{0} and N\textsubscript{100} indicating the presence of significant variability among genotypes. Moderate PCV, GA with moderate high heritability observed for most of the traits including dry biomass under N\textsubscript{0} suggesting that family/pedigree selection in large population (Roy, 2000) suitable for trait selection under nitrogen deficient condition.
Table 1: Details of prebred genetic stocks used in the study

| Genotypes | Clone       | Parentage                                                                 | Salient Features                          |
|-----------|-------------|----------------------------------------------------------------------------|--------------------------------------------|
| G1        | Co 07017    | PIR 83-327 x Co 86011                                                      | Commercial cane                            |
| G2        | Co 06021    | Co 7201 x 97-257                                                          | Commercial cane                            |
| G3        | Co 07001    | Co 86011 x PIO 90-188                                                      | Commercial cane                            |
| G4        | Co 93009    | Co 678 x Co 775                                                           | Commercial cane                            |
| G5        | CoC 671     | Co 775 x Q63                                                               | Commercial cane                            |
| G6        | Co 94019    | Co 7201 x Co 62175                                                        | Commercial cane                            |
| G7        | Co 94012    | Somaclone of CoC 671                                                      | Commercial cane                            |
| G8        | Co 95020    | Co 7407 x (CP 44101 x NG 7794)                                            | Commercial cane                            |
| G9        | Co 06002    | Co 85002 x OH 44                                                           | Commercial cane                            |
| G10       | Co 07004    | Co 85002 x 96-77                                                          | Commercial cane                            |
| G11       | ISH 04-2097 | Co 8371 (2n=108) x SH 216 (2n=72)                                          | Interspecific hybrids of S. spontaneum     |
| G12       | ISH 479     | BO 130 (2n=110) x IND 82-228 (2n=40)                                      | Interspecific hybrids of S. spontaneum     |
| G13       | ISH1757     | Co 86249 (2n=108) x SES 590 (2n=64)                                        | Interspecific hybrids of S. spontaneum     |
| G14       | ISH732      | Co 1148 (2n=114) x IND 82-319 (2n=56)                                     | Interspecific hybrids of S. spontaneum     |
| G15       | ISH1875B    | Co 89029 (2n=110) x IND 84–394(2n=112)                                    | Interspecific hybrids of S. spontaneum     |
| G16       | ISH04-941   | Co 8371 2n=108) x IND 84–415 (2n=80)                                      | Interspecific hybrids of S. spontaneum     |
| G17       | GU 07-3488  | GU04(22)RE560 x Co 775                                                    | BC1 of E. arundinaeous                     |
| G18       | GU 07-5317  | GU (50)RE-16 X CoS 510                                                     | BC1 of E. arundinaeous                     |
| G19       | GU 04(72) COE-1 | CoC 671 x IK 76-91                                      | IGH of E. arundinaeous                     |
| G20       | GU 07-2276  | GU 04 (50) RE-9 X CoH 70                                                  | BC1 of E. arundinaeous                     |
| G21       | CYM 12-509  | CYM 10-601xCoT 8201                                                       | Fourth generation hybrids of S. spontaneum x E. arundinaeous with sugarcane. |
| G22       | CYM 12-450  | CYM 10-601xCoT 8201                                                       | Fourth generation hybrids of S. spontaneum x E. arundinaeous with sugarcane. |
| G23       | CYM 12-447  | CYM 10-601xCoT 8201                                                       | Fourth generation hybrids of S. spontaneum x E. arundinaeous with sugarcane. |
| G24       | CYM 12-456  | CYM 10-601xCoT 8201                                                       | Fourth generation hybrids of S. spontaneum x E. arundinaeous with sugarcane. |
| G25       | CYM 12-476  | CYM 10-601xCoT 8201                                                       | Fourth generation hybrids of S. spontaneum x E. arundinaeous with sugarcane. |
| G26       | GU 12-25    | GU04(28)EO-2 x Co 06027                                                   | BC1 progeny of E. procerus                 |
| G27       | GU 12-35    | GU04(28)EO-2 x Co 06027                                                   | BC1 progeny of E. procerus                 |
| G28       | GU 12-27    | GU04(28)EO-2 x Co 06027                                                   | BC1 progeny of E. procerus                 |
| G29       | GU 12-38    | GU04(28)EO-2 x Co 06027                                                   | BC1 progeny of E. procerus                 |
| G30       | GU 12-60    | GU04(28)EO-2 self                                                         | Self-progeny of E. procerus                |
| G31       | GU 12-12    | SSH 27 x Co 94008                                                          | BC 1of Saccharum-Sorghum hybrid            |
| G32       | GU 07-5622  | SSH-1 x CoC 8001                                                          | BC 1of Sorghum- Saccharum hybrid           |
Table.2 Estimates of genetic variability parameters for traits associated with agronomic use efficiency of nitrogen under N₀ and N₁₀₀ condition

| Traits                          | PCV   |       | GCV   |       | Heritability (BS) |       | GAM   |       |
|--------------------------------|-------|-------|-------|-------|-------------------|-------|-------|-------|
|                                | N₀    | N₁₀₀  | N₀    | N₁₀₀  | N₀    | N₁₀₀  | N₀    | N₁₀₀  |
| No. of tillers                 | 31.96 | 35.85 | 14.72 | 30.17 | 0.21  | 0.71  | 13.97 | 52.31 |
| Stalk height (cm)              | 15.58 | 10.31 | 10.81 | 5.92  | 0.48  | 0.33  | 15.46 | 7.00  |
| Stalk diameter (cm)            | 16.44 | 16.24 | 14.23 | 14.07 | 0.75  | 0.75  | 25.37 | 25.11 |
| Single stalk weight (kg)       | 37.79 | 33.27 | 32.00 | 28.61 | 0.72  | 0.74  | 55.81 | 50.70 |
| Number of stalks               | 36.73 | 42.29 | 29.61 | 39.11 | 0.65  | 0.86  | 49.16 | 74.52 |
| Juice brix (%)                 | 17.75 | 19.77 | 19.41 | 20.94 | 0.84  | 0.89  | 33.42 | 38.46 |
| Sucrose (%)                    | 28.89 | 33.60 | 26.70 | 31.83 | 0.85  | 0.90  | 50.85 | 62.12 |
| Purity (%)                     | 15.13 | 18.91 | 14.21 | 18.18 | 0.88  | 0.92  | 27.49 | 35.99 |
| CCS (%)                        | 33.76 | 40.47 | 31.28 | 38.34 | 0.86  | 0.90  | 59.71 | 74.81 |
| Extraction (%)                 | 14.06 | 13.65 | 12.88 | 13.20 | 0.84  | 0.93  | 24.31 | 26.28 |
| Fibre (%)                      | 19.18 | 23.46 | 18.84 | 23.25 | 0.96  | 0.98  | 38.11 | 47.45 |
| Dry Matter (%)                 | 7.17  | 10.26 | 6.11  | 9.67  | 0.73  | 0.89  | 10.74 | 18.78 |
| Fresh biomass yield (t/ha)     | 37.85 | 37.10 | 20.98 | 30.75 | 0.31  | 0.69  | 23.96 | 52.51 |
| Dry biomass yield (t/ha)       | 37.52 | 34.06 | 22.58 | 28.92 | 0.36  | 0.72  | 28.01 | 50.60 |
Table 3 Mean performances of Co canes, ISH and IGH clones derived from *E. arundinaceus*, *E. procerus* and Sorghum for traits associated with agronomic use efficiency of nitrogen under N₀ and N₁₀₀ condition

| Traits                        | Nitrogen Levels | Genotypes     |
|-------------------------------|----------------|---------------|
|                               | N₀       | N₁₀₀      | ‘Co’ canes | ISH       | IGEA       | IGEP       | IGSS       |
| No. of tillers                |          |           |           |           |           |           |            |
| N₀                            | 133.80** | 148.55** | 116.67    | 113.80    | 174.07**  | 216.67**  | 123.46     | 125.62     |
| N₁₀₀                          |           |           | 212.50**  | 243.50**  | 221.25**  | 260.83**  | 238.61     | 239.44     |
| Stalk height (cm)             | 226.64** | 247.50** | 212.50**  | 243.50**  | 221.25**  | 260.83**  | 238.61     | 239.44     |
| Stalk diameter (cm)           | 2.37**   | 2.41**   | 2.52      | 2.61      | 1.87      | 2.01      | 2.57       | 2.60       |
| Single stalk weight (kg)      | 0.75**   | 0.88**   | 0.82*     | 1.02*     | 0.44*     | 0.65*     | 0.92**     | 0.94**     |
| Number of stalks              | 103.04** | 122.45** | 80.46     | 84.44     | 152.78**  | 195.68**  | 90.74**    | 106.28**   |
| Juice brix (%)                | 17.90**  | 16.57**  | 20.75*    | 19.67*    | 13.03**   | 11.55**   | 18.51**    | 16.63**    |
| Sucrose (%)                   | 15.30**  | 13.65**  | 18.96*    | 17.75*    | 8.96**    | 6.62**    | 16.12**    | 13.86**    |
| Purity (%)                    | 83.22**  | 79.50**  | 91.17     | 89.87     | 64.75**   | 54.46**   | 86.44**    | 82.89**    |
| CCS (%)                       | 10.41**  | 9.11**   | 13.31*    | 12.40*    | 5.36**    | 3.40**    | 11.07**    | 9.31**     |
| Extraction (%)                | 45.96**  | 47.96**  | 47.64**   | 51.02**   | 38.26**   | 42.03**   | 48.80**    | 51.41**    |
| Fibre (%)                     | 18.85**  | 17.79**  | 16.98**   | 15.71**   | 23.75**   | 21.66**   | 16.88**    | 15.85**    |
| Dry Matter (%)                | 33.46**  | 31.49**  | 34.22**   | 32.31**   | 33.70**   | 30.73**   | 32.28**    | 29.86**    |
| Fresh biomass yield (t/ha)    | 108.04** | 144.15** | 93.44     | 114.99    | 107.25**  | 188.13**  | 116.69     | 139.43     |
| Dry biomass yield (t/ha)      | 35.00**  | 46.54**  | 31.18*    | 37.65*    | 35.96**   | 57.78**   | 36.09*     | 44.73*     |

* Significant at 5% level, ** significant at 1% level
Fig. 1 Agronomic NUE in commercial clones, Interspecific and intergeneric hybrids of sugarcane
Gascho et al., (1986) reported that nitrogen use efficiency of sugarcane can be enhanced by selection under low N condition inferred based on quantum of nitrogen accumulated in different varieties in low nitrogen condition.

Agronomic traits and biomass attributing traits (fresh and dry biomass yield) recorded significantly higher mean in N₁₀₀ and juice quality parameters (brix %, sucrose %, CCS %), fibre% and dry matter % recorded significantly higher mean in N₀. Similar reports of nitrogen deficient condition enhancing the accumulation of sucrose was reported in sugarcane cultivars (Kumar and Bandara, 2002), SP80-3280 (Rhein et al., 2016) and Q117 (Muchow et al., 1996). The maximum AgNUE in the interspecific hybrids of S. spontaneum and clones with E. procerus base had shown that these species could be the better sources for development nitrogen use efficient varieties in sugarcane. One of the important findings of the study is the early generation hybrids of S. spontaneum (F₁), E. procerus (BC₁) had higher AgNUE than the later generation clones. It clearly indicates limited variation in modern cultivars (Co canes) and necessitates utilization of wild relatives in improving NUE in sugarcane. Hawkesford (2017) has also reported a significant and limited variation for NUE and suggested for broader germplasm for improving NUE in wheat. The interspecific hybrids ISH 732, ISH 1875B, ISH 04-941, Erianthus procerus hybrid derivatives GU 12-25, GU 12-60, intergeneric hybrid derivatives with E. arundinaceus CYM 12-450, GU 07-5622, GU 04(72) COE-1, GU 07-2276 and commercial clones Co 95020 and Co 07004 recorded maximum AgNUE could be the potential sources for developing nitrogen efficient varieties in sugarcane (Fig 1). Therefore, these genotypes further considered for utilization in crop improvement programmes for development of elite breeding pools for nitrogen use efficiency.

**Future line of works**

Development of nitrogen use efficient responsive sugarcane varieties required to reduce the emission of greenhouse gases, conservation of aquatic ecosystem and cost benefit the farmers. Evaluation of genetically diverse genotypes consisting of improved ‘Co’ canes and prebreeding material derived from progenies of S. spontaneum, E. arundinaceus, E. procerus and Saccharum-sorghum hybrids under N₀ and N₁₀₀ showed the presence of genetic variability for traits attributing to nitrogen use efficiency. Genotypes were categorized into nitrogen use efficient responsive, nitrogen use non responsive, nitrogen use inefficient responsive and nitrogen use inefficient non responsive genotypes. Therefore, nitrogen use efficient responsive genotypes used for crop improvement programmes through conventional and marker assisted selection. Indian sugarcane varieties and germplasms shall be screened for nitrogen use efficiency and catalogued as nitrogen use efficient and responsiveness. Novel biotechnological approaches such as transcriptome sequencing for gene identification and transgenic approaches for trait improvements. Besides many rhizosphere nitrogen fixing and endogenous microbes × genotypes shall have been characterized for identification nitrogen use efficient nitrogen responsive genotypes.

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