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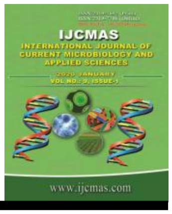

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Genetic Variability for Nitrogen Use Efficiency in Interspecific and Intergeneric Hybrids of Sugarcane

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ABSTRACT

Keywords

Sugarcane, Agronomic nitrogen use efficiency (AgNUE)

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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, 32diverse sugarcane pre-breeding genetic stocks were evaluated with two levels of nitrogen (N_0 and N_{100}) 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 (N_0 and N_{100}) 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.

Introduction

Sugarcane is an importantC_4 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 26.52mhawith 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 $kgha^{-1}a$ and low in P (27.30 kg ha^{-1}) and high in K (718.06 $kgha^{-1}$).

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 N_0 and recommended dose of Nitrogen (280kgha^{-1}) designated as N_{100}.

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).

$$AgNUE = \frac{\text{Dry biomass under N 100} - \text{Dry biomass under N0}}{\text{Quantity of Nitrogen applied}}$$

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 (N_0 and N_{100}) 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 N_0 and N_{100} is presented in table 2.Higher range, GCV and PCV for most of agronomic and biomass attributing traits was recorded in $N_{100}$and for juice quality parameters in N_0. Wider differences were observed between GCV and PCV indicating the role of nitrogen levels (N_0 and N_{100}) 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 $N_{100}$and low heritability of 0.21, 0.31 and 0.36 under N_0respectively.

Performance of agronomic traits at N_0 and N_{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 $N_{100.}$ Juice quality parameters such as brix %, sucrose %, juice purity %, CCS (%), fibre(%) and dry matter (%) recorded significantly higher mean under $N_{0.}$

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 trashes (Dias *et al.*, 2011; Furlan *et al.*, 2013; Smithers, 2014; Leal *et al.*, 2016). It is a high nitrogen demanding cropand 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_2O) 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_2O 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_0 and N_{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_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. arundinaceus*
G18	GU 07-5317	GU (50)RE-16 X CoS 510	BC1 of *E. arundinaceus*
G19	GU 04(72) COE-1	CoC 671 x IK 76-91	IGH of *E. arundinaceus*
G20	GU 07-2276	GU 04 (50) RE-9 X CoH 70	BC1 of *E. arundinaceus*
G21	CYM 12-509	CYM 10-601xCoT 8201	Fourth generation hybrids of *S.spontaneum x E. arundinaceus* with sugarcane.
G22	CYM 12-450	CYM 10-601xCoT 8201	Fourth generation hybrids of *S.spontaneum x E. arundinaceus* with sugarcane.
G23	CYM 12-447	CYM 10-601xCoT 8201	Fourth generation hybrids of *S.spontaneum x E. arundinaceus* with sugarcane.
G24	CYM 12-456	CYM 10-601xCoT 8201	Fourth generation hybrids of *S.spontaneum x E. arundinaceus* with sugarcane.
G25	CYM 12-476	CYM 10-601xCoT 8201	Fourth generation hybrids of *S.spontaneum x E. arundinaceus* 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 progenyof *E. procerus*
G28	GU 12-27	GU04(28)EO-2 x Co 06027	BC1 progenyof *E. procerus*
G29	GU 12-38	GU04(28)EO-2 x Co 06027	BC1 progenyof *E. procerus*
G30	GU 12-60	GU04(28)EO-2 self	Self-progenyof *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 N_0 and N_{100} condition

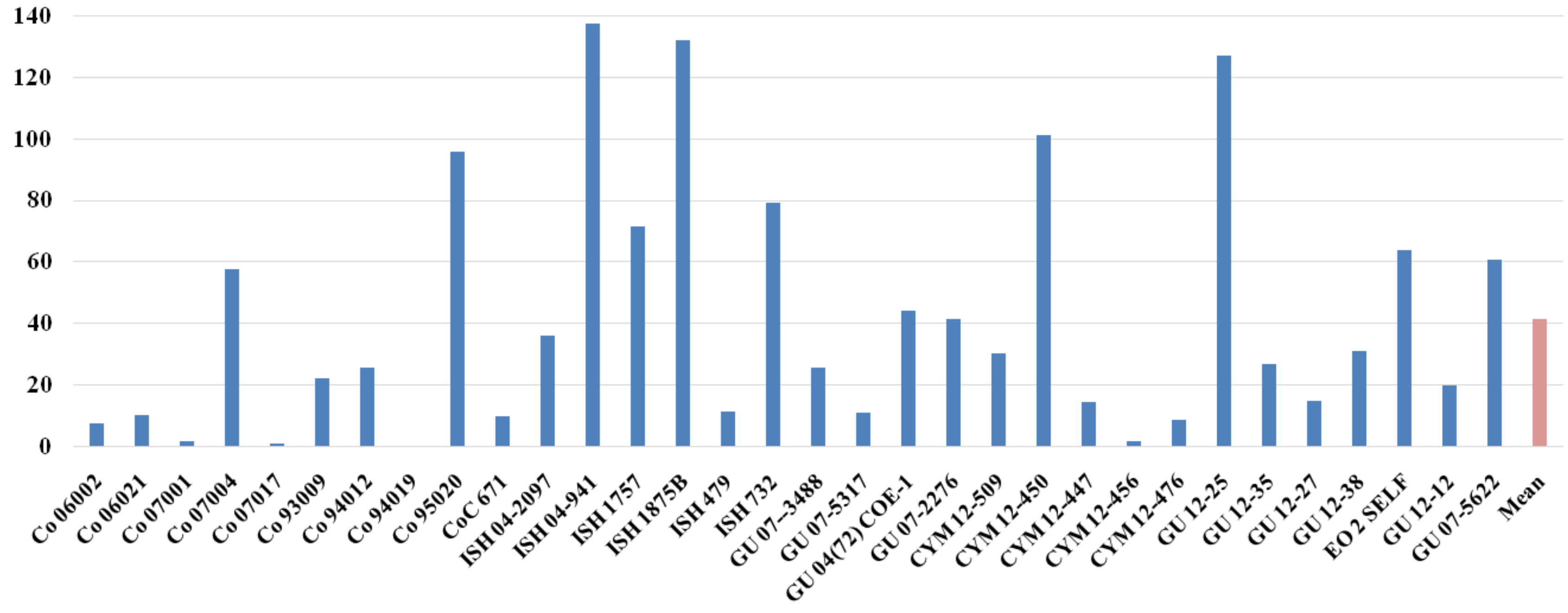
Traits	PCV		GCV		Heritability (BS)		GAM	
	N0	N100	N0	N100	N0	N100	N0	N100
No. of tillers	31.96	35.85	14.72	30.17	0.21	0.71	13.97	52.31
Stalk height (cm)	15.58	10.31	10.81	5.92	0.48	0.33	15.46	7.00
Stalk diameter (cm)	16.44	16.24	14.23	14.07	0.75	0.75	25.37	25.11
Single stalk weight (kg)	37.79	33.27	32.00	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 N_0 and N_{100} condition

Traits	**Nitrogen Levels**		**Genotypes**									
			'Co' canes		**ISH**		**IGEA**		**IGEP**		**IGSS**	
	N0	**N100**	**N0**	**N100**	**N0**	**N100**	**N0**	**N100**	**N0**	**N100**	**N0**	**N100**
No. of tillers	133.80**	148.55**	116.67	113.80	174.07**	216.67**	123.46	125.62	131.67	158.33	150.46**	196.76**
Stalk height (cm)	226.64**	247.50**	212.50**	243.50**	221.25**	260.83**	238.61	239.44	222.50*	248.00*	270.00	262.50
Stalkdiameter (cm)	2.37**	2.41**	2.52	2.61	1.87	2.01	2.57	2.60	2.25	2.19	2.48*	2.30*
Single stalk weight (kg)	0.75**	0.88**	0.82*	1.02*	0.44*	0.65*	0.92**	0.94**	0.60	0.71	0.97	0.95
Number of stalks	103.04**	122.45**	80.46	84.44	152.78**	195.68**	90.74*	106.28*	101.30**	124.44**	126.39**	160.65**
Juice brix (%)	17.90**	16.57**	20.75*	19.67*	13.03**	11.55**	18.51**	16.63**	17.50*	16.54*	16.49	15.99
Sucrose (%)	15.30**	13.65**	18.96*	17.75*	8.96**	6.62**	16.12**	13.86**	14.65	13.70	13.89	13.11
Purity (%)	83.22**	79.50**	91.17	89.87	64.75**	54.46**	86.44**	82.89**	83.41	81.85	83.92	81.56
CCS (%)	10.41**	9.11**	13.31*	12.40*	5.36**	3.40**	11.07**	9.31**	9.86	9.17	9.38	8.72
Extraction (%)	45.96**	47.96**	47.64**	51.02**	38.26**	42.03**	48.80**	51.41**	45.04**	42.60**	50.21*	48.39*
Fibre (%)	18.85**	17.79**	16.98**	15.71**	23.75**	21.66**	16.88**	15.85**	20.17	20.67	19.05**	18.12**
Dry Matter (%)	33.46**	31.49**	34.22**	32.31**	33.70**	30.73**	32.28**	29.86**	34.17**	33.77**	32.40*	31.22*
Fresh biomass yield (t/ha)	108.04**	144.15**	93.44	114.99	107.25**	188.13**	116.69	139.43	97.26**	130.02**	171.51**	214.52**
Dry biomass yield (t/ha)	35.00**	46.54**	31.18*	37.65*	35.96**	57.78**	36.09*	44.73*	31.23**	45.97**	55.65**	66.90**

* 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 N_{100} and juice quality parameters (brix %, sucrose %, CCS %), fibre% and dry matter % recorded significantly higher mean in $N_{0.}$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* (F_1), *E.procerus* (BC_1) 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 N_0 and N_{100} 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 hall 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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