

Full Length Research Paper

Guttation fluid as a physiological marker for selection of nitrogen efficient rice (*Oryza sativa* L.) genotypes

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A field experiment was conducted during the rainy season of 2008 and 2009 to use guttation fluid as a physiological marker for the screening of more nitrogen efficient rice genotypes on the basis of relationship between Guttation Fluid (GF) oozed by leaf tip and nitrogen use efficiency (NUE), grain yield (GY) and biological yield (BY) amongst five rice (KRH-2- hybrid, Kasturi- aromatic, Krishna Hamsa, Tulsı and Vasumati- high yielding) genotypes grown at four nitrogen levels (0, 50, 100 and 200 kg ha⁻¹) in alluvial soil of Pantnagar (Uttarakhand), India. The nitrogen fertilizer (urea) was sprouted in the field. For this experiment, the field was made to keep with 5 cm standing water throughout active tillering and reproductive stage. Guttation fluid (GF) was collected during flowering stage whilst other traits after harvesting of the crops. The utmost and lowest GF was achieved by genotype KRH-2 and Kasturi respectively. All the rice genotypes showed the positive correlation between GF and NUE, GY, BY at different nitrogen levels vice-versa. The KRH-2 illustrated better response to secretion of guttation fluid and other traits. The experiment concluded that the amount of GF is directly associated with application of fertilizer as well as NUE, GY and BY. Further studies are good opportunities for rice researcher to improve rice yield through this way and mapping the genes controlling this trait and creating rice plant with increase guttation fluid at different nitrogen levels for selection of high nitrogen efficient rice genotypes.

Key words: Biological yield, guttation fluid, nitrogen use efficiency, grain yield, rice genotypes.

INTRODUCTION

Rice is one of the most important crops of the world and forms the staple diets of about 2.7 billion people and it needs to be produced 50% more than what is produced now by 2050 to cope with the growing demand (Ashikari et al., 2005). In Asia, 90% of the world's rice is produced and consumed. It is very well known fact that stability and growth in current and future scenario of every nation is only achievable by using their resources efficiently and effectively to meet the demand of rapidly growing populations (Ahmad et al., 2009). The yield of rice is an integrated result of various processes, including canopy

photosynthesis, conversion of assimilates to biomass, partitioning of assimilates to grains (harvest index) and interception of light by leaf surface area. Grain weight is an important yield component in cereal crops. It is determined by the source capacity (photosynthetic leaves) to supply assimilate during the ripening period, and by sink capacity (developing grain) to accumulate the imported assimilate. Cultivars with larger grain size tend to have higher grain filling rate, resulting in higher assimilate accumulation and heavier grain weight (Kropff et al., 1994; Zhang et al., 2004; Yoshida et al., 2006;

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Table 1. Some basic properties of soil from experimental site.

Soil properties	Values
Soil colour	Dark grayish brown to dark grey
Texture	Loam to silty clay loam
Soil water (dry mass percentage of water)	13.99 to 19.78 (June to September)
Temperature of soil	26 to 32°C (June to September)
Bulk densities	1.10 to 1.46 mg m ⁻³ (June to September)
Hydraulic conductivity	317 to 407 mm h ⁻¹
Infiltration rate	269 to 624 mm h ⁻¹
pH	6.74 to 8.05
EC	0.33 to 0.60 dSm ⁻¹
CaCO ₃	0.42 to 0.87%
CEC	8.1 to 18.22 meq 100 g ⁻¹ soil
Organic carbon	0.39 to 1.61%
Zn	0.17 to 2.11 mg kg ⁻¹
Cu	0.64 to 2.49 mg kg ⁻¹
Mn	1.31 to 45.69 mg kg ⁻¹
Fe	3.09 to 21.41 mg kg ⁻¹
Total N	0.14%
Available P	44.28 ppm
Available K	280.59 kg ha ⁻¹

Yang et al., 2005). In addition to this, nitrogen is the key factor which limits the yield of rice production around the world. The predominant form of nitrogenous fertilizer applied to the soil is urea. In agriculture, 60% nitrogen is used for cultivation of cereal crops alone, out of 60% approximate 30% of applied N is utilized by crops and the remaining 70% of it is lost through various process causing serious environmental problems (Jiang et al., 2005). Thus, efficient utilization of nitrogen fertilizers is essential for ensuring better yield as well as to minimize the adverse impacts of nitrogen species in the environment.

Nitrogen use efficiency varies amongst rice genotypes due to genetic variation in uptake of nitrogen. High nitrogen application increased the nitrogen uptake but if the application exceeds optimum value, the yield decreased (Wilson et al., 2006). The NUE values ranged from 35.6 to 51.6% for different genotypes (Samonte et al., 2006). Various strategies are being used to enhance yield of the rice crop worldwide such as the transfer of C₄ trait to C₃ plants for improving the photosynthetic performance of C₃ plants (Miyao, 2003). Beside this, another alternative approach for improving rice yield is the selection of varieties those secreting more amount of guttation fluid which is non-invasive, simple, accurate and quick to perform. Guttation fluid is also a potential yield enhancing trait in rice. This guttational fluid contained various organic and inorganic substances of metabolic significance. Ozaki and Tai (1962) found three peptides in the guttation liquid of rice seedling and considered that peptides might be predominant form of nitrogen transport in the

xylem of rice seedling. The rates of guttation fluid positively correlated with their panicle weight or grain yield productivity (Singh et al., 2008, 2009).

The rice leaves secrete guttation fluid through openings situated along the leaf margin and tips called hydathodes. The rates of guttation exhibited by various cultivars were positively correlated with their panicle weights, that is, yield sink potential, which is a direct measure of grain yield productivity. The amount of guttation fluid is directly associated with yield of rice crop. The hybrid and heigh yielding rice genotypes show better response to secretion of guttation fluid from leaf tips (Singh et al., 2008, 2009). Now its time to use guttation fluid as a physiological marker for screening of more nitrogen use efficient as well maximizing grain yield to avoid maximum application of nitrogen fertilizer for cultivation of rice crop. Therefore, this study will be benefitted in future for selection of high yield and more nitrogen efficient genotypes using guttation fluid as a physiological marker.

MATERIALS AND METHODS

The experiment was carried out at Dr. N. E. Borlaug's Crop Research Center (29° N latitude, 79° 29' E longitude and at an altitude of 243.8 msl.), Department of Plant Physiology, G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India, during *khariif* season 2008 and 2009 with five rice genotypes, namely KRH-2 (hybrid), Kasturi, Krishna Hamsa, Tulsi (high yielding) and Vasumati (aromatic) with four nitrogen levels (0, 50, 100 and 200 kg N ha⁻¹). Some basic properties of soil from experimental site are shown in Table 1. The field experiment was laid out in a split plot design with 3 replications. The sub-plot size

Table 2. Effect of N levels on guttation fluid ($\mu\text{l}/\text{tip}$) of five rice genotypes.

Genotype	Guttation fluid ($\mu\text{l}/\text{tip}$)				
	Flowering stage				
	N ₀	N ₅₀	N ₁₀₀	N ₂₀₀	Mean
KRH-2	97	110	135	163	126
Vasumati	95	106	119	146	116
Kasturi	90	104	122	142	114
Tulsi	93	108	125	150	119
Krishna Hamsa	101	114	130	154	124
Mean	95	108	126	151	

	Treatment (T)	Variety (V)	T × V
S.E.M. \pm	0.29	0.33	0.67
CD ($\rho = 5\%$)	1.01	0.97	1.95

was 4 × 4 m. The 25 days old seedlings were transplanted with 20 cm row space and at 10 cm plant to plant distance. Nitrogen fertilizer in the form of urea was sprouted in three splits as 50% at 15 days after transplanting, 25% at panicle initiation and remaining 25% at the time of flowering stage. The field was made to keep with 5 cm standing water during active tillering and reproductive stage. The bund (approximate 1 m thick and 0.7 m height) was prepared around entire plot as well as sub plots to avoid the movement of applied fertilizer and water to adjacent plots.

The guttation fluid from the flag leaf of individual genotypes at flowering stage was achieved using method described by Singh et al. (2008) and amount expressed in $\mu\text{l}/\text{tip}$. Little modifications were made in this method. The Hamilton syringe and eppendorf tubes were used to collect guttation fluid from the leaf tip instead of blotting paper used in the original method. The guttation fluid from the flag leaf of individual genotypes at flowering stage was collected in eppendorf with help of Hamilton syringe. The fluid was collected during daylight between 04:00 to 06:00 pm to avoid merge of dew some time deposited on the leaves. Before collecting the droplets, leaf was trickled down into eppendorf from exuding sites. The amount of fluid exuded in volumetrically was determined through the help of Hamilton syringe and amount expressed in $\mu\text{l}/\text{tip}$. The nitrogen use efficiency was calculated as the ratio of the grain yield (kg grain ha^{-1}) to the N applied (kg N ha^{-1}). Grain yield from each plot was recorded and finally expressed as g m^{-2} . For calculation of biological yield, each plant was uprooted from ground level at maturity and then bundled and labeled and after drying, the weight of intact plant was determined before threshing and the total weight of the plant was recorded as biological yield in g m^{-2} . The correlation between guttation fluid, NUE, grain yield and biological yield vice versa was made. The data was analyzed statistically for calculating standard error of mean (SEM) and critical difference (CD) at 5% probability level (Panse and Shukhtme, 1978). The data shown in tables are the mean of two years for 2008 and 2009.

RESULTS AND DISCUSSION

Guttation fluid

The guttation fluid (GF) which oozed through the leaf tip of rice genotypes was directly affected with application of N fertilizer (Table 2). The amount of guttation fluid (GF)

drastically increased from N₀ level to N₂₀₀ and maximum achieved at N₂₀₀ level of nitrogen applied. It is interesting to know that the applied nitrogen fertilizer exhibit positive correlation with guttation fluid (GF) for all rice genotypes. The maximum guttation fluid at flowering stage was observed for KRH-2 while the minimum for Kasturi followed by Tulsi, Vasumati and Kasturi. The reasons behind it, the KRH-2 is a hybrid rice genotype that has more capacity to extract available nitrogen from the soil and it showed better response to nitrogen fertilizer at different nitrogen levels. In addition to this, the hybrid and high yielding rice genotypes have more ability to guttate more amount of guttation fluid than inbred and low yielding rice varieties. Similar results were revealed by other researchers (Singh et al., 2008, 2009).

Nitrogen use efficiency

Nitrogen use efficiency also showed a significant correlation among applied nitrogen fertilizer (Table 3) and also exhibited positive relationship with guttation fluid (Figure 1). The nitrogen use efficiency was increased with increasing N levels up to N₁₀₀ level (Table 3). Further increase in N levels decreased the NUE. It is due to that, at higher concentration of N the absorption exceeds the utilization. The KRH-2 was found most N efficient genotype whereas Kasturi least efficient. In field experiments, hybrid rice had a greater N efficiency than conventional rice (Lin et al., 1980). It was reported that NUE was increased from N₄₅ (20.00), up to N₉₀ (31.00) but further increase in N levels decreased nitrogen use efficiency as N₁₃₅ (29.00) and N₁₈₀ (19.00). Nitrogen use efficiency is a genotypic parameter and ranged from 35.6 to 51.6 (kg grain kg^{-1} N absorbed) for different genotypes (Samonte et al., 2006). The current average nitrogen use efficiency (NUE) in the field is approximately 33% and substantial proportion of the remaining 67% is lost into the environment, especially

Table 3. Effect of N levels on NUE of five rice genotypes.

Genotype	Nitrogen use efficiency (%)				Mean
	N ₀	N ₅₀	N ₁₀₀	N ₂₀₀	
KRH-2	28.35	70.26	56.50	39.33	48.61
Vasumati	21.13	22.15	19.91	19.43	20.66
Kasturi	16.94	23.63	20.04	19.64	20.06
Tulsi	25.31	30.55	24.93	25.18	26.49
Krishna Hamsa	25.27	44.26	34.03	33.65	34.30
Mean	23.40	38.17	31.08	27.45	

	Treatment (T)	Variety (V)	T × V
S.E.M. ±	0.49	0.39	0.79
CD ($p = 5\%$)	1.70	1.14	2.29

Table 4. Effect of N levels on grain yield of five rice genotypes.

Genotype	Grain Yield (g m ⁻²)				Mean
	N ₀	N ₅₀	N ₁₀₀	N ₂₀₀	
KRH-2	311.00	759.33	768.00	610.00	612.08
Vasumati	259.00	291.67	300.00	296.67	286.84
Kasturi	229.67	341.67	283.33	300.67	288.84
Tulsi	301.00	383.33	383.33	408.33	369.00
Krishna Hamsa	301.00	583.33	508.33	541.67	483.58
Mean	280.33	471.87	448.60	431.47	

	Treatment (T)	Variety (V)	T × V
S.E.M. ±	24.09	21.71	43.56
CD ($p = 5\%$)	83.25	62.55	125.11

cropped areas (Abrol et al., 2007).

Grain yield

The significant relationship between grain yield, applied nitrogen fertilizer and guttation fluid was recorded. The grain yield was found to be increased with increasing N doses and highest recorded at N₂₀₀ level for most of the genotypes. But for some rice genotypes like Kasturi (147.67) and Krishna Hamsa (123.67), it was highest at N₅₀ level than N₁₀₀ and for rice genotype Krishna Hamsa (131.00); it was maximum at N₅₀ levels than N₅₀ and N₁₀₀ levels (Table 4). The KRH-2 got maximum grain yield followed by Krishna Hamsa, Tulsi, Kasturi and Vasumati. This increased grain yield due to increases grain number in response to application of N fertilizers and enhanced availability of N. Besides this, number of grains per panicle was significantly influence by application of nitrogen fertilizer (Manzoor, et al., 2006; Zhenxie et al., 2008).

Biological yield (g m⁻²)

The biological yield is the result of several physiological as well as biochemical process and also depends on which types of genotype are used. It is noted that biological yield and guttation fluid showed significant relation with applied nitrogen fertilizer and vis-à-vis (Table 5 and Figure 1). The results of biological yield showed the similarity as the grain yield. It was utmost recorded for hybrid rice genotype KRH-2 and the least for rice genotype Tulsi (Table 5). Similar results were observed by Manzoor (2006). More straw yield could be a result of more biomass and it can be due to higher capability of hybrid rice to utilize more N through the expression of better growth by accumulating more dry matter (Meena et al., 2003).

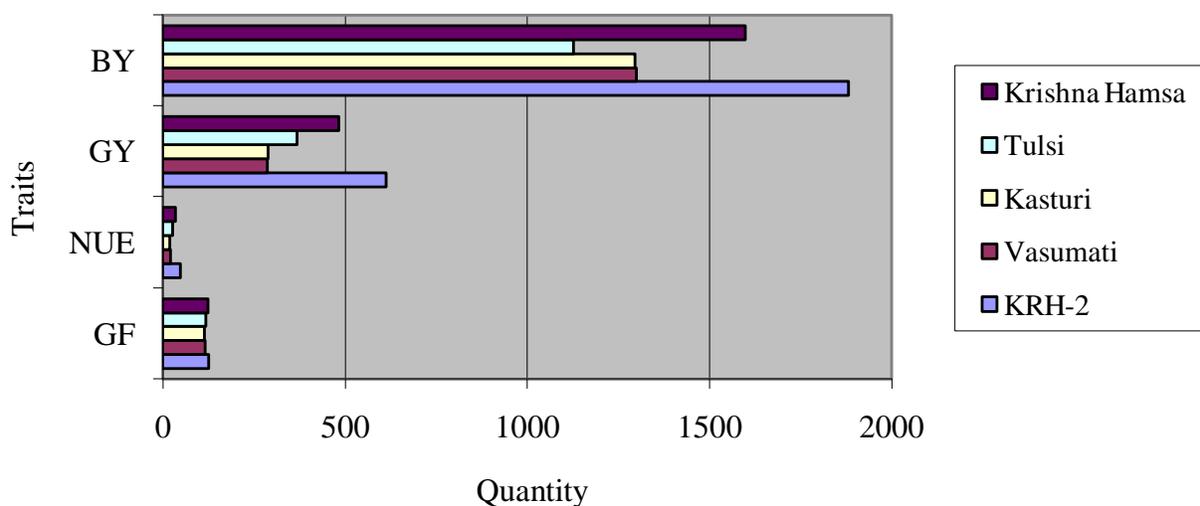
Relationship between GF, NUE, GY and BY

It was too interesting to observe that the yield related traits such as nitrogen use efficiency, grain yield and

Table 5. Effect of N levels on biological yield of five rice genotypes.

Genotype	Biological yield (g m ⁻²)				Mean
	N ₀	N ₅₀	N ₁₀₀	N ₂₀₀	
KRH-2	955.67	1757.67	2408.83	2402.33	1881.13
Vasumati	858.00	1341.52	1401.00	1596.83	1299.34
Kasturi	835.83	1526.80	1381.55	1441.83	1296.50
Tulsi	783.93	1283.48	1141.83	1295.50	1126.19
Krishna Hamsa	991.57	1851.00	1675.83	1876.23	1598.66
Mean	885.00	1552.09	1601.81	1722.54	

	Treatment (T)	Variety (V)	T × V
S.E.M. ±	33.28	37.20	74.62
CD (p = 5%)	115.03	107.16	214.32

**Figure 1.** Relationship between Guttative Fluid-GF (µl/tip) and Nitrogen Use Efficiency-NUE (%), Grain Yield-GY (g m⁻²), Biological Yield-BY (g m⁻²) amongst five genotypes of rice.

biological yield illustrated positive correlation with guttation fluid and vice-versa. In addition to this, strong positive correlation was observed between guttation fluid (GF), nitrogen use efficiency (NUE), grain yield (GY) and biological yield (BL) amongst five rice genotypes treated with four nitrogen doses (Figure 1). The genotype showed greater amount of guttation fluid oozed through the leaf tip, same time got maximum NUE, grain yield and biological yield too. Out of five rice genotypes, the rice genotype KRH-2 gained more guttation fluid and NUE followed by Krishna Hamsa, Tulsi, Vasumati and Kasturi. The reasons behind it is that the KRH-2 is a hybrid nature genotype having better response to nitrogen fertilizer and also because of more capacity to extract N and guttate more amount of guttation fluid than inbred and low yielding rice varieties. It was interesting to know that these findings were supported by other researcher they observe that the higher the volume of exuded fluid by the

varieties, the greater their panicle weights and grain yield (Singh et al., 2008, 2009).

Conclusion

A positive correlation was concluded between guttation fluid (GF) and nitrogen use efficiency (NUE), grain yield (GY) and biological yield (BY) for all rice genotypes namely KRH-2, Kasturi, Krishna Hamsa, Tulsi and Vasumati treated with N₀, N₅₀, N₁₀₀ and N₂₀₀ kg ha⁻¹ levels. Besides this, based on the yield traits, it was also concluded that KRH-2 rice genotype revealed more efficiency to nitrogen uptake due to secretion of more guttation fluid. Further, guttation fluid can be used as a physiological marker for screening high yielding rice varieties for high nitrogen use efficiency. Secretion of guttation fluid by leaf tip is directly associated with better

uptake of nitrogen and nitrogen use efficiency and amount of guttation fluid enhanced by application of different levels of nitrogen fertilizer. Hence, this study offer good opportunity for rice researcher to improve rice yield through this way and mapping the genes controlling this trait and producing rice plant with increase guttation fluid at different N levels.

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REFERENCES

- Jiang L, Dong D, Gan X, Wei S (2005). Photosynthetic efficiency and nitrogen distribution under different nitrogen management and relationship with physiological nitrogen use efficiency in three rice genotypes. *Plant Soil*. 271: 321-328.
- Wilson LT, Medley JC, Lales JS (2006). Nitrogen utilization efficiency: Relationship with grain yield, grain protein and yield related traits in rice. *Agron. J*. 98: 168-176.
- Samonte RK, Wilson LT, Medley JC, Pinson SRM, McClung M, Lales JS (2006). Nitrogen utilization efficiency: Relationship with grain yield, grain protein and yield related traits in rice. *J. Agron*. 98: 168-176.
- Ozaki K, Tai K (1962). Nitrogen constituents of Guttation in paddy rice plant. *Bull. Fac. Agri. Mie Univ. Japan*. 26: 171-176.
- Singh S, Chauhan JS, Singh TN (2008). Guttation: A potential yield enhancing trait in rice. *Curr. Sci*. 95: 455-456.
- Singh S, Singh TN, Chauhan JS (2009). Guttation in rice: occurrence, regulation, and significance in varietal improvement. *J. Crop Improv*. 23: 351-365.
- Panse VG, Shukhtme PV (1978). Statistical methods for agricultural workers. IARI, New Delhi.
- Lin SC, Yuan LP (1980). Hybrid rice breeding in China. *In: Innovative approaches to rice breeding*. Manila (Philippines). *Int. Rice Res. Ins.*, pp: 35-37.
- Abrol YP, Raghuram N, Sachdev MS (2007). Agricultural nitrogen use and its environmental implications. I. K. International, New Delhi, pp: 552.
- Manzoor Z, Ali RI, Awan TH, Khalid N, Ahmad M (2006). Appropriate time of nitrogen application to fine rice (*Oryza sativa*). *J. Agric. Res*. 44: 261-267.
- Zhenxie Y, Wang P, Tao H, Zhang H, Shen L (2008). Effects of types and application rates of nitrogen fertilizer on the development and nitrogen utilization of summer maize. *Front. Agric. China*. 2: 44-49.
- Meena SL, Surendra S, Shivay YS, Singh S (2003) Response of hybrid rice (*Oryza sativa*) to nitrogen and potassium application in sandy clay loam soils. *I. J. Agric. Sci*. 73: 8-11.
- Ashikari MH, Sakakibara S, Lin T, Yamamoto, Takashi T (2005). Cytokinin oxidase regulates rice grain production. *Sci*. 309:741-745.
- Ahmad S, Ahmad A, Zia-ul-Haq M, Ali H, Anjum MA, Khan MA, Khaliq T, Hussain A, Hoogenboom G (2009). Resources use efficiency of field grown transplanted rice (*Oryza sativa* L.) under irrigated semiarid environment. *J. Food, Agri. Environ*. 7: 487-492.
- Kroff MJ, Cassman KG, Peng S, Setter TL, Matthews RB (1994). Quantitative understanding of rice yield potential. *In: KG. Cassman (Editor), breaking the yield barrier*. International Rice Research Institute, Los Banos, Philippines, Pp: 21-38.
- Zhang SX, Li XY, Li XP, Yuan FM, Yao ZH, Sun YL et al. (2004). Crop yield, N uptake and nitrates in a fluvo-aquic soil profile. *Pedosphere*. 14: 131-136.
- Yang XG, Bouman BAM, Wang HQ, Wang ZM, Zhao JF, Chen B (2005). Performance of temperate aerobic rice under different water regimes in North China. *Agric. Water Manage*. 74: 107-122.
- Yoshida H, Horie T, Shiraiwa T (2006). A model explaining genotypic and environmental variation of rice spikelet number per unit area measured by cross-locational experiments in Asia. *Field Crops Res*. 97: 337-343.
- Miyao M, Fukayama H (2003). Metabolic consequences of overproduction of phosphoenolpyruvate carboxylase in C_3 plants. *Arch. Biochem. Biophys*. 414:197-203.