PERFORMANCE OF SUGARCANE (*Saccharum officinarum* L.) MUTATION PLANT BY ETHYL METHANE SULPHONATE (EMS) AT SEVERAL DOSES OF NITROGEN APPLICATION

Sri Hartatik*, Miswar, Tirto Wahyu Widodo

Faculty of Agricuture, University of Jember, Jalan Kalimantan 37, Jember
Telp/fax: (0331) 334054/ (0331) 338422
*Corresponding Author: srihartatik1@yahoo.com

ABSTRACT

The increased of national sugar production can be done by planting high sugar content varieties, that had been created by mutation. The large amount of sugar content related to sucrose biosynthesis capacity. The previous research reported that mutant of sugarcanes cv. Bululawang had 15.5- 18.5% sugar content but non-mutant ones had only 10.81% sugar content. The aimed of this research was obtaining the optimum dose of N that reached highest sugar content in every mutant of sugarcanes. Research was conducted in Jember village from August 2016 until June, 2017. The experiment was arranged in Completely Randomized Design with two factors and three replications. First factor was composed of non-mutant of sugarcane cv. Bululawang (M2.1) and three mutant of sugarcane (M2.2, M2.3, M2.4), while second factor was five levels of nitrogen i.e 350 kg N/ha (N0), 385 kg N/ha (N1), 420 kg N/ha (N2), 455 kg N/ha (N3) and 495 kg N/ha (N4). The result showed that mutant of sugarcanes needed N supply lower than non-mutant ones. Optimum dose of N for obtaining high cane sucrose content in mutant of sugarcanes cv. Bululawang M2.2 (17,61 %), M2.3 (16,51 %), and M2.4 (15,74 %) were 403,79 kg N ha^{-1}, 392,35 kg N ha^{-1}, and 301,93 kg N ha^{-1}, respectively. Optimum N dose for non-mutant of sugarcane cv. Bululawang (10,98 %) was 414,18 kg N ha^{-1}. Stem sucrose content (sugar content), leaf sucrose content, stalk and leaf reducing sugar, stalk diameter, and internode length were higher in mutant of sugarcanes than non-mutant ones.

Keywords: nitrogen application, plant mutation, sucrose content, sugarcane

BACKGROUND

Indonesian sugar production decreased every year. The sugar production was only 2.49 million tons, while the consumption reached 5.7 million tons in 2015. The sugar production was related to sugar content (sucrose). The low of sugar production was caused by sugar content. Indonesian sugar content average in 2015 was only 8.28%.

Improvement of sugar content in sugarcane always be done through crossing, genetic transformation, and mutation. Plant mutation was one method to change genetic composition, so the mutated plant had difference characters. Genetic mutation could be done through chemical and physical mutation. The result of research by Miswar *et al.* (2016) using EMS proved that mutant of sugarcanes cv. Bululawang had 15.5-18.5% sugar content but non-mutant ones had only 10.81% sugar content. Mutated sugarcane that had high of sugar

content consists of 3 genotypes, ie genotype with 18.58%, 16.83%, and 15.57% sugar content.

The genetic difference between mutant and non-mutant of sugarcane causes nutrition difference needed, especially nitrogen (N). N is one of organic nutrient that helps biosynthesis and accumulation of sucrose in sugarcane. Sugarcane cv. Bululawang needed 207 kg N ha^{-1} (Naruputro & Purwono, 2009). Wijaya (2016) reported that sugarcane cv. Bululawang needed 350 kg N ha^{-1} with absorbed 210 kg N ha^{-1}. Mutants of sugarcane are estimated requiring different N. The application of N is expected to determine the optimum dose for mutants of sugarcane before release.

MATERIALS AND METHODS

The research was conducted in Jember from August, 2016 until June, 2017. The physiological characters were analyzed in Plant Analysis Laboratory, Agriculture Faculty, Jember University.

Materials used are non-mutant (M2.1) and mutant (M2.2, M2.3, M2.4) of sugarcane cv. Bululawang that was obtained from experiment of Miswar *et al.* (2016), nitrogen source (ZA 21% N), SP-36, KCl and chemicals needed in the analysis of physiological caharacters.

Experiment was arranged in Completely Randomized Design (CRD) with two factors and three replications. First factor was composed of non-mutant (M2.1) and three mutant of sugarcane (M2.2, M2.3, M2.4), while second factor was five levels of nitrogen i.e 350 kg N ha^{-1} (N0), 385 kg N ha^{-1} (N1), 420 kg N ha^{-1} (N2), 455 kg N ha^{-1} (N3) and 495 kg N ha^{-1} (N4).

Cheking soil N content was performed before planting sugarcane, where the soil N content was 113.93 kg N ha^{-1}. Applied N dose based on treatment minus available soil N. Two-month-old bud chip of sugarcane seedling was planted at 50x75 cm^2 polybags. Fertilizer applied two times, 1/3 of N dose at initial planting and the rest of dose at two months after planting. Tissues analysis was performed on nine-month-old sugarcane.

The variables observed are sugar content in term of stalk sucrose, leaf sucrose, leaf reducing sugar, stalk diameter, internode length, number of internode, and number of tiller. Sucrose and reducing sugar content were determined according to Miswar (2007) and Miswar (2001), respectively.

RESULTS AND DISCUSSION

The differences nitrogen needed in genotypes of sugarcane caused determining of high sugar content. Sugarcane mutant required different N optimum dose to reach the highest sugar content like 7.77 g N per polybag (403.79 kg N ha^{-1}) for M2.2, 7.55 g N/polybag (392.35 kg N ha^{-1}) for M2.3, and 5.81 g N/polybag (301.93 N ha^{-1}) for M2.4, while M1 (non mutant) required N optimum dose 7.97 g N/polybag (414.18 kg N ha^{-1}), see the picture 1. Contrary to that, Hemalatha (2015) reported that the highest sucrose content was obtained in the treatment which received N 195.5 kg N ha^{-1} and sucrose content decreased when N dose was increased.

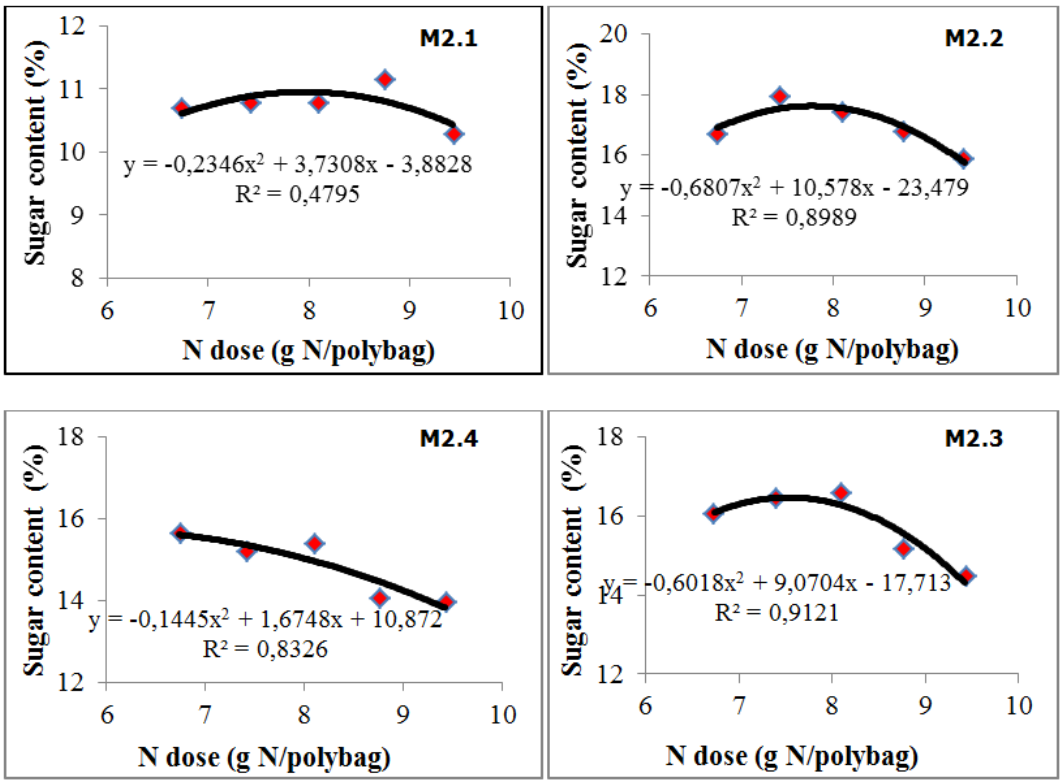

Figure 1. Regression Analysis of sugar content in non mutant and mutant of sugarcanes

Table 1. Summary of mean square in several observed variables

No	Variables	Mean Square			
		Genotypes (M)	N Dose (N)	M x N	Error
1	Sugar content	108.53**	4.40 ns	0.66 ns	1.93
2	Leaf sucrose	5.24**	0.09 ns	0.04 ns	0.13
3	Stalk reducing sugar	1.32ns	0.75 ns	0.79 ns	0.89
4	Leaf reducing sugar	1.19 ns	2.27 ns	0.51 ns	1.57
5	Stalk diameter	0.11**	0.01 ns	0.02 ns	0.02
6	Internode length	1.78*	0.96 ns	0.50 ns	0.93
7	Number of internode	0.16 ns	0.16 ns	0.10 ns	0.16
8	Number of tiller	1.09 ns	2.35*	1.28 ns	0.74

*,** = significantly different; ns = not-significantly different

Picture 1 showed that increasing N dose (M2.1, M2.2, and M2.3) could increased sucrose content until the high point then the sucrose content would turn down. In this case, excessive N absorption caused plants containing a high water (succulent), so the sucrose content of sink tissues decreased. N would stimulated cell division and cell enlargement rapidly so as to produce cells containing a high water and thin-walled (Marschner, 1995). Studies reported that excess application of N on sugarcane could decreased the sucrose content (Muchow *et al.,* 1996; Hemalatha, 2015). Higher N rate might caused the cane growth period to be longer and sugar accumulation period to be shorter. A better balance was achieved with a medium fertilizer dose (Koochekzadeh *et al.,* 2009). According to picture 1, M2.4 needed 5.81 g N/polybag (301.93 N ha^{-1}) to obtain the highest sucrose content. M2.4 was thought to be able to absorb and use N

efficiently, so the optimum dose under the dose used in this study. Contrary to that, Sharma and Gupta (1991) reported that nitrogen use efficiency in sugarcane was significantly higher at higher fertilizer levels.

Table 2. Characters of non-mutant and mutant of sugarcanes

No	Variables	Genotypes			
		M2.1	M2.2	M2.3	M2.4
1	Sugar content	10.73 b	16.93 a	15.73 a	14.83 a
2	Leaf sucrose	1.89 b	3.00 a	3.15 a	3.06 a
3	Stalk reducing sugar	4.81	4.99	5.13	4.45
4	Leaf reducing sugar	10.79	11.43	11.33	11.17
5	Stalk diameter	2.63 a	2.72 a	2.67 a	2.52 b
6	Internode length	15.22 b	15.54 b	15.34 b	16.01 a
7	Number of internode	10.96	11.11	11.20	11.04
8	Number of tiller	6.58	7.39	7.10	7.14

Dunnette Test (0.05): Means in each variable followed by the same letters are not-significantly different from each other

Sucrose would be accumulated in sugarcane stalk. Sucrose accumulation in sugarcane depended on variety and planting technique. In this study, sucrose content in stalk and leaf was observed in nine-month-old non-mutant of sugarcane (M2.1) and mutant of sugarcanes (M2.2, M2.3, M2.4) based on 5% Dunnett test, see the picture 2. The highest leaf sucrose content was M2.3, then M2.4, M2.2, and M2.1. Sucrose content in sugarcane stalk had positive correlation with sucrose content in leaf. Mutant of sugarcanes (M2.2, M2.3, M2.4) had higher sugar content than non-mutant ones (M2.1), see picture 3. Khan *et al.* (2007) reported that induced mutation with gamma irradiation (40 Gy) caused sucrose content of sugarcanes mutant was higher than non-mutant of sugarcanes cv. BL4 and NIA98. Genetic variability on sugarcane caused differences sucrose metabolism, so sucrose accumulation was also different.

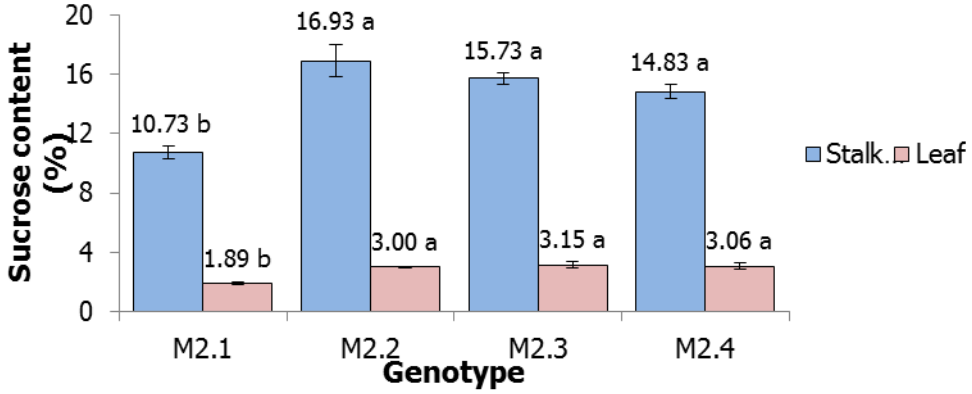

Figure 2. Stalk and leaf sucrose content in non mutant (M2.1) and mutant (M2.2, M2.3, M2.4) of sugarcanes

Sucrose accumulation in stalk tissues depended on sucrose biosynthesis and degradation process in leaf, so sucrose content in stalk related to difference between SPS and invertase enzymes activity (Huber & Huber, 1992; Zhu *et al*., 1997). Increasing of sucrose degradation was followed by increasing reducing sugar, so sucrose content of sugarcane related to reducing sugar content.

Sugarcane leaf that had saturated sucrose would increased sucrose metabolism activity for creating balance condition. If leaf sucrose accumulation was much, it would increased sucrose degradation and transportation. If sucrose content was high, so the reducing sugar content would be lower and vice versa (Erwinda & Susanto, 2014).

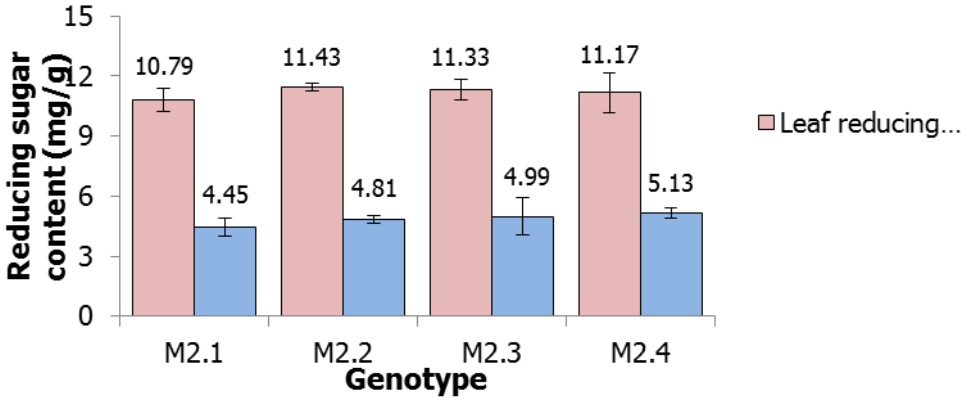

Figure 3. Stalk and leaf reducing sugar content in non mutant (M2.1) and mutant (M2.2, M2.3, M2.4) of sugarcanes

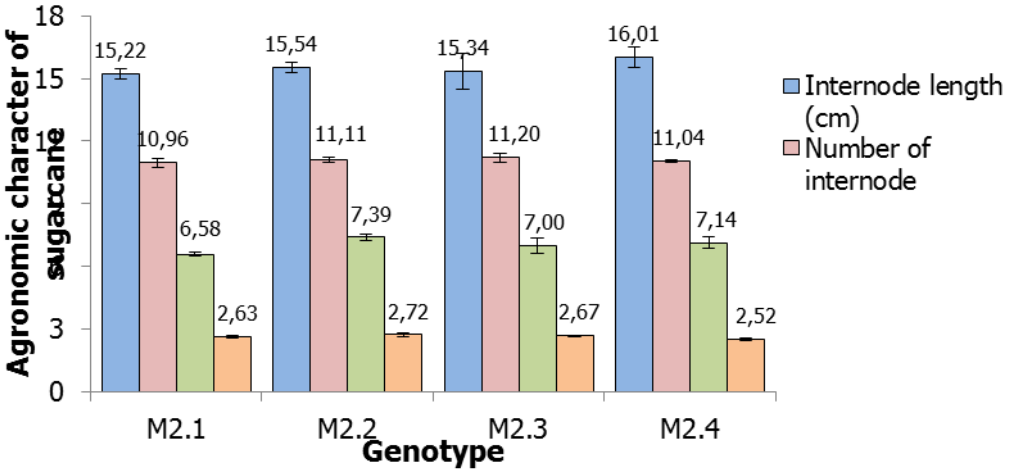

Figure 4. Agronomic character of non mutant (M2.1) and mutant (M2.2, M2.3, M2.4) of sugarcanes

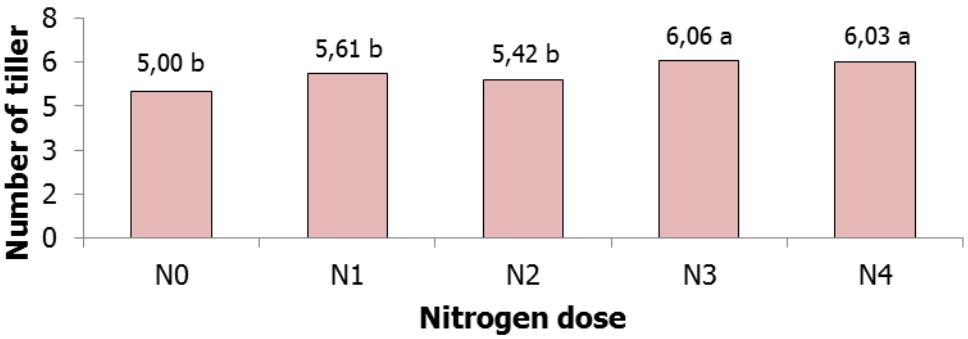

Figure 5. Number of cane tiller on several nitrogen dose

The result of this study reported that leaf and stalk reducing sugar content was not significantly different in M2.1, M2.2, M2.3, dan M2.4, see picture 3. Pereira *et al.* (2007) reported that reducing sugar content was similar during maturation, although there were differences in the level of sugar in sink tissues of sugarcane. Generally, mutant of sugarcanes had higher leaf reducing sugar content than non-mutant ones. It was happened because there was relation with sucrose hydrolyzed enzymes activity. If the sucrose hydrolyzed enzymes activity increased, so the reducing sugar product would be higher. Indriani *et al.* (2015) reported that invertase was one of sucrose hydrolyzed enzyme that reduced sucrose to glucose and fructose (reducing sugar).

Sucrose on young tissues would be hydrolyzed into glucose and fructose that used as respiration substrate. Plants utilize cell repiration results for their metabolic processes, such as cell division and elongation. Mutant of sugarcanes had larger internode length and number of internode than non mutant-ones (Figure 4), although the internode length of the M2.1 was not significantly different with M2.2 and M2.3 based on the 5% Dunnett test. Mutant of M2.4 had the longest internode length compared to other sugarcane, but the internode diameter was smallest (Figure 4). Mutant of M2.4 was thought to have a great ability of cells elongation that were showed by the internode length, but the cell enlargement was small. Mutants of M2.2 and M2.3 tended to have larger stem diameters compared to non-mutants, although it did not significantly difference. Mutant of sugarcane also had the ability to form more tillers than non-mutant ones, see Figure 5.

CONCLUSION

1. Mutant of sugarcanes needed lower N supply than non mutant-ones to reach highest sugar content.
2. The optimum dose of N for obtaining high sugar content were 414.18 kg N ha^{-1}, 403.79 kg N ha^{-1}, 392.35 kg N ha^{-1}, and 301.93 kg N ha^{-1} for M2.1, M2.2, M2.3 and M2.4, respectively.
3. Sugar content, leaf sucrose content, stalk and leaf reducing sugar, stalk diameter and internode length of sugarcanes mutant disposed be higher than non mutant-ones.

REFERENCES

Erwinda, M.D., W.H. Susanto. (2014). Pengaruh pH nira tebu (*Saccharum officinarum*) dan konsentrasi penambahan kapur terhadap kualitas gula merah. Pangan dan Aroindustri, 2(3): 54-64.

Gomathi, R., P.N.G. Rao, P. Rakkiyappan, B.P. Sundara, S. Shiyamala. 2013. Physiological studies on ratoonability of sugarcane varieties under tropical indian condition. American Journal of Plant Sciences. 4: 274-281.

Hemalatha, S. 2015. Impact of nitrogen fertilization on quality of sugarcane under fertigation. IJRSI. 2(3): 37-39.

Huber, J.L., S.E. Huber. 1992. Role of sucrose phosphate synthase in sucrose metabolism in leaf. Plant Physiol. 99:1275-1278.

Indriani, D.O., L.N.I. Syamsudin, F.H. Sriherfyna, A.K. Wardani. 2015. Invertase dari *Aspergillus niger* dengan metode *solid state fermentation* dan aplikasi di industri: kajian pustaka. Pangan dan Agroindustri. 3(4):1405-1411.

Khan, I.A., M.A. Dahot, A. Khatri. 2007. Study of variability in sugarcane induced through mutation breeding. Pak. J. Bot. 39(5): 1489-1501.

Koch, K. 2004. Sucrose Metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Plant Biology. 7(1):235-246.

Koochekizadeh, A., G. Fahi, M.H. Gharineh, S.A. Siadat, S. Jafari, Kh. Alami-Saeid. 2009. Impacts of rate and split application of N fertilizer on sugarcane quality. Inter. J. Agric. Res. 4:116-123.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, New York, USA.

Miswar. 2001. Aktifitas enzim metabolisme sukrosa dan perubahan sintesis protein tanaman kedelai *(Glycine max* (l.) Merr*.)* pada kondisi cekaman garam (NaCl) tinggi*.* Laporan Penelitian*.* Jember: Universitas Jember.

Miswar. 2007. Peningkatan biosintesis sukrosa tanaman tebu (*Saccharum officinarum* L.) melalui over ekspresi gen sucrose phosphate synthase (SPS). Disertasi. Yogyakarta: Universitas Gadjah Mada.

Miswar, D.E. Munandar, U. Solikhah. 2016. Perakitan tanaman tebu (S*accharum officinarum* L.) rendemen tinggi melalui mutasi DNA secara kimiawi untuk mendukung program swasembada gula pemerintah. Laporan Akhir Penelitian Strategis Nasional. Jember: Universitas Jember.

Muchow, R.C., M.J. Robertson. 1994. Relating crop nitrogen uptake to sugarcane yield. Proceedings of Australian Society of Sugarcane Technologists.

Naruputro, A., Purwono. 2009. Pengelolaan tanaman tebu (*Saccharum officinarum* L.) di pabrik gula Krebet Baru, PT. PG. Rajawali I, Malang, Jawa Timur; dengan aspek khusus mempelajari produktivitas pada tiap kategori tanaman. Makalah Seminar Departemen Agronomi dan Hortikultura. Bogor: Institut Pertanian Bogor.

Pereira, L.F.M., V.M. Ferreira, N.G. De Oliveira, P.L.V.S. Sarmento, L. Endres, I. Teodoro. 2017. Sugars levels of four sugarcane genotypes in different stem portions during the maturation phase. Annals of the Brazilian Academy of Sciences, 89(2): 1231-1242.

Sharma, O.L., P.C. Gupta. 1991. Effect of levels of irrigation, N and methods of planting on N uptake and dry matter production of sugarcane. Ind. Sugar. 41: 611-613.

Wijaya, K.A. 2016. Effects of Si-fertilizer application through the leaf on yield and sugar content of sugarcane grown in soil containing abundant N. Agriculture and Agricultural Science Procedia. 9:158-162.

Zhu, Y.J., E. Komor, P.H. Moore. 1997. Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. Plant Physiol. 11 (5):609-616.