IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 7, Issue 10 Ver. I (Oct. 2014), PP 47-55
www.iosrjournals.org

Response of Stevia (*Stevia rebaudiana* Bertoni M) To Nitrogen and Potassium Fertilization

Inugraha[1], M. D. Maghfoer[2] and E. Widaryanto[2]
[1](*Master Program of Plant Science, Faculty of Agriculture, University of Brawijaya, Malang)*
[2](*Faculty of Agriculture, University of Brawijaya, Malang)*

Abstract: *Objective of this research was to obtain optimal dosages of nitrogen and potassium fertilization on growth, yield, and quality of stevia. The research was conducted using Randomized Block Design (RBD) by 3 replications, which was constructed in factorial that comprised of 2 factors. The first factor was dosage of the nitrogen with 4 (four) levels, such as: 100, 150, 200, and 250 kg N ha^{-1}. The second factor was dosage of potassium with 3 (three) levels, such as: 75, 150, and 225 kg K_2O ha^{-1}. Result of the research showed that significant interaction between nitrogen and potassium fertilization has occurred on parameter of the plant growth, such as number of leaf, leaf area, leaf area index, and the total chlorophyll content. Separate application of nitrogen and potassium fertilization has significantly affected on the plant height, while the stomatal density was only affected by the application of nitrogen. Significant interaction between nitrogen and potassium application has occurred on plant biomass and the yield of leaf during the harvest period I, II, and total. During the period of total harvest, the fertilizing treatments of 200 kg N ha^{-1} and 225 kg K_2O ha^{-1} have harvested fresh leaves and dry leaves for about 2,780 kg ha^{-1} and 636 kg ha^{-1}, respectively. The increasing application of nitrogen from 100 to 250 kg N ha^{-1} has significantly increased the stevioside level of the stevia's leaf linearly in accordance with the equation of $Y = 0.000408 X + 0.439$.*
Keywords: *Stevia, nitrogen, potassium, fertilization, growth, yield*

I. Introduction

Benefit of stevia that contains stevioside has been well-known and mostly used as substitution of sucrose (cane sugar) sweetener as well as synthetic sweeteners (saccharine, cyclamate, aspartame, asulpham-K), due to not only safe but also beneficial for health. Stevia has been well-known in Indonesia since 1977, however, cultivation of the stevia is relatively less developed at present. Based on the information, it is presumed that production of stevia in Indonesia has still lower, as well as lack of cultivation technology on stevia, therefore the development of stevia in Indonesia has not optimal as expected.

Growth, yield, and quality of stevia are not only affected by genetic factor, but also environment, and one of them is nutrient availability for the plant. The main nutrients required by the plant were nitrogen and potassium. Nitrogen is an essential nutrient that could improve the growth during the vegetative phase and protein synthesis. According to Hardjowigeno [1], nitrogen is applied in plant, which is going to be taken the leaves, because it would make the foliars grow well. Sufficient nitrogen in the soil would make the plants look greener, which mean that nitrogen plays its role in forming chlorophyll for photosynthetic process.

In particular, potassium plays in physiological and metabolism process of the plant, such as regulating the respiration through stomata, enzymatic activities in forming starch, increasing resistance to drought and diseases [1], therefore it would affect on growth and translocation of the photosynthetic products among the plant's tissues. Besides that, potassium could improve quality of the plant, such as increasing the contents of starch, oil, or other secondary metabolite compounds.

Based on few scientific reviews on response of the nitrogen and potassium to growth, yield, and quality of stevia in Indonesia, this research must be done and as expected that it would be able to provide scientific information to support the development of stevia in Indonesia.

II. Material And Method

The research was conducted at the Experiment Farm (EF) of Cangar, Faculty of Agriculture, University of Brawijaya, in Sumberbrantas Village, Bumiaji Subdistrict of Batu, East Java, at the altitude of 1,660 meter above sea level (asl) at daily temperature of 19.9-21.3^{o}C, on the average. Analysis of the physical and chemical characteristics of the soil before planting is presented in Table 1. The research was conducted from November 2013 to May 2014 using Randomized Block Design (RBD) by 3 replications, which was constructed in factorial that comprised of 2 factors. The first factor was dosage of the nitrogen with 4 (four) levels, such as: 100, 150, 200, and 250 kg N ha^{-1}. The second factor was dosage of potassium with 3 (three) levels, such as: 75, 150, and 225 kg K_2O ha^{-1}.

Table 1. Physical and chemical characteristics of soil at EF Cangar

Parameter	Value	Status
Soil texture	Sandy clay	
- Sand (%)	57	
- Dust (%)	35	
- Clay (%)	8	
pH H_2O	7.21	Medium
C-organic (%)	3.72	High
N total (%)	0.32	Medium
Ratio C/N	11.63	Medium
P_2O_5 Olsen (ppm)	26.3	Very high
K (me)	1.48	Very high

The planting materials are cuttings of *Stevia rebaudiana* Bert. cultivar Super Green of 25 days derived from Tawangmangu of Central Java, chicken manure, Ammonium Sulphate or ZA [$(NH_4)_2SO_4$], SP-36 and KCl. Chicken manure 10 t ha^{-1} was applied during soil cultivation. Phosphate (75 kg P_2O_5 ha^{-1} equivalent with 1.89 g SP-36 $plant^{-1}$) and potassium (in accordance with treatment) and 50% N (in accordance with treatment) were applied at 7 Days After Transplanting (DAT), the remains of N was applied at 30 DAT. For ratoon plant, fertilization of P, K (in accordance with treatment) and 50% N (in accordance with treatment) was done at 2 days after harvest time and the remains of N was applied at 30 days after harvest time.

Observation on growth during the first planting period includes: plant height (cm), number of leaf $plant^{-1}$, leaf area (cm^2), Leaf Area Index (LAI), total chlorophyll content in leaf, and stomatal density. Observation at the first harvest was done to measure the yield components (root, stem, and leaf $plant^{-1}$) as well as harvest yield (leaf and stem). For observation during the ratoon period was only done on the harvest yield (leaf and stem). Analysis on stevioside level in leaf was done at the first harvest time in composite using Thin Layer Chromatography (TLC). Analysis on leaf tissues and the second analysis on soil were done during the harvest time ratoon plants in composite in order to find out the content and adsorption of N, P, and K by the plants as well as the nutrient remains in the soil.

Data of observation was analyzed by analysis of variance (F-test) in the level of 5% to find out the effect of treatment. If the result had significant differences, it will be followed by LSD (Least Significant Difference) test in the significant level of 5% to find out the difference among treatment.

III. Result And Discussion

1.1 Plant Height

Plant height during the period of growth has shown no significant interaction between the application of nitrogen and potassium. Fertilization level of nitrogen has significant effect on plant height at 30 to 90 DAT. Furthermore, the fertilization level of potassium has significant effect on plant height at 30 to 90 DAT (Table 2). Maheswar [2] reported that the increasing dosage of nitrogen from 60 kg N ha^{-1} to 105 kg N ha^{-1} could increase height of stevia for about 16.01% (from 48.96 into 56.8 cm). Aladakatti et al. [3] also reported that the increasing dosage of nitrogen from 200 to 400 kg N ha^{-1} could increase height of stevia for about 14.94% (from 49.59 into 57 cm).

1.2 Number of Leaf

Significant interaction occurred between the application of nitrogen and potassium on number of leaf $plant^{-1}$ at 30 and 60 DAT. At 90 DAT, number of leaf $plant^{-1}$ is just only affected separately by the application of nitrogen and potassium (Table 2). Maheswar [2] reported that the increasing dosage of nitrogen from 60 kg N ha^{-1} to 105 kg N ha^{-1} could increase number of leaf $plant^{-1}$ for about 49.13% (from 304.77 into 454.50 blade $plant^{-1}$). Aladakatti et al. [3] also reported that the increasing dosage of nitrogen from 200 to 400 kg N ha^{-1} could increase number of leaf $plant^{-1}$ for about 12.97% (from 750.4 into 847.7 blade $plant^{-1}$).

1.3 Leaf Area

Significant interaction occurred between the application of nitrogen and potassium on leaf area $plant^{-1}$ at 30 and 60 DAT. At 90 DAT, leaf area $plant^{-1}$ is just only affected separately by the application of nitrogen and potassium (Table 2). Maheswar [2] reported that the increasing dosage of nitrogen from 60 kg N ha^{-1} to 105 kg N ha^{-1} could increase leaf area $plant^{-1}$ for about 83.81% (from 3,083.12 into 5,667.23 cm^2). Aladakatti [4] also reported that the increasing dosage of nitrogen from 200 to 400 kg N ha^{-1} could increase leaf area $plant^{-1}$ for about 15.88% (from 3,628 into 4,204 cm^2).

1.4 Leaf Area Index

Significant interaction occurred between the application of nitrogen and potassium on leaf area index at 30 and 90 DAT (Table 2). Leaf area index increased during the early growth phase and reached the peak at 60

DAT, and then reduced at 90 DAT. It can be explained through an approach as stated by Gardner et al. [5] that the increasing rate of leaf area has run slowly due to leaf shading below it, which affected photosynthesis on the related leaves. On the contrary, the increasing rate of leaf area above it has run faster due the absorbed light for photosynthesis was more spread evenly, so that it affected on wider area that covered the ground.

Table 2. The Effect of Nitrogen (N) and Potassium (K_2O) on Average Height (H), Number of Leaf (NL), Leaf Area (LA), Leaf Area Index (LAI) at 30, 60, and 90 DAT

Treatment (kg ha^{-1})		30 DAT				60 DAT				90 DAT			
N	K_2O	H (cm)	NL (blade plant^{-1})	LA (cm^2)	LAI	H (cm)	NL (blade plant^{-1})	LA (cm^2)	LAI	H (cm)	NL (blade plant^{-1})	LA (cm^2)	LAI
100	75	11,42	12,33	41,00	0,97	20,83	24,67	172,33	1,84	38,83	117,67	432,33	0,66
	150	12,60	13,33	35,33	0,87	21,83	33,00	187,00	1,80	41,17	142,00	466,00	0,74
	225	14,33	12,67	78,67	1,38	25,67	42,33	242,17	1,93	44,67	166,33	516,83	1,11
150	75	13,33	11,67	64,33	1,22	23,83	34,67	215,67	1,79	45,83	176,00	533,50	0,88
	150	15,50	14,00	95,67	1,53	29,00	57,00	290,50	1,81	51,83	245,33	620,17	1,36
	225	16,33	15,67	94,17	1,32	32,83	81,33	354,00	1,69	52,33	254,67	641,58	0,95
200	75	15,33	13,67	93,00	1,54	24,33	38,33	223,00	1,85	41,67	140,00	473,33	0,85
	150	18,75	19,67	142,50	1,72	29,83	66,00	302,67	2,05	53,83	264,00	648,83	1,24
	225	14,17	12,00	76,33	1,41	23,67	34,00	213,33	1,84	47,50	192,33	557,49	0,91
250	75	13,67	12,67	69,00	1,21	22,50	29,67	196,67	1,87	44,00	161,67	507,00	0,81
	150	17,93	18,00	130,67	1,71	23,00	43,33	204,00	1,83	43,83	158	504,67	0,96
	225	16,17	15,00	105,00	1,59	28,83	56,00	287,83	1,96	50,17	219,33	596,33	1,10
N		*	*	*	*	*	*	*	ns	*	*	*	*
K_2O		*	*	*	*	*	*	*	ns	*	*	*	*
N x K_2O		ns	*	*	*	ns	*	*	ns	ns	ns	ns	*
CV (%)		11,77	12,83	24,30	13,16	12,71	23,66	19,39	14,23	11,40	24,43	14,13	14,31
LSD 5% N		1,72	-	-	-	3,17	-	-	-	5,16	44,53	74,19	-
LSD 5% K_2O		1,49	-	-	-	2,75	-	-	-	4,47	38,57	64,77	-
LSD 5% N x K_2O		-	3,09	35,17	0,31	-	18,04	79,05	-	-	-	-	0,23

Notes: DAT = Days After Transplanting, ns = non significant, * = significant difference in level 5%, CV = Coefficient of Variance, LSD = Least Significant Difference

1.5 Total Chlorophyll Content in Leaf

Significant interaction occurred between the application of nitrogen and potassium on total chlorophyll content in leaf at 60 DAT (Table 3). Total chlorophyll content in leaf of each treatment of nitrogen and potassium application has increased till 90 DAT, even though the significant difference could be seen statistically at 60 DAT. During the observation, potassium played more dominant role in increasing total chlorophyll in leaf than nitrogen. Of course, it related to the role of potassium in physiological process of the plant, such as photosynthetic rate, transpiration rate, and stomatal conductivity [6]. As stated by Lei Ma and Yan Shi [7] that the chlorophyll content in stevia's leaf has increased along with the increasing dosage of potassium in comparison with without potassium application.

On the other side, the content level of chlorophyll during the application of nitrogen was highly related to N adsorption level of the plant. Even though N adsorption by the leaf was high along with the increasing dosage of nitrogen (Table 7), however, it is assumed that higher N adsorption by the plant was not only used to form chlorophyll, but a part of them was also allocated to other meristem, which was not identified in the observation.

Table 3. The Effect of Nitrogen (N) and Potassium (K_2O) on the Average of Total Chlorophyll Content in Leaf

Treatment (kg ha^{-1})		Total Chlorophyll Content (mg g^{-1} dw)		
N	K_2O	30 DAT	60 DAT	90 DAT
100	75	3.16	4.75	4.63
	150	3.14	3.93	4.41
	225	3.05	4.36	4.93
150	75	3.30	3.88	4.43
	150	3.38	4.58	4.37
	225	3.76	4.70	4.89
200	75	3.53	4.00	4.61
	150	3.16	4.10	4.75
	225	3.67	4.79	4.61
250	75	3.51	3.96	4.65
	150	3.54	4.41	5.04
	225	3.45	4.74	5.12
N		ns	ns	ns
K_2O		ns	*	ns

N x K_2O	ns	*	ns
CV (%)	14.61	9.26	9.66
LSD 5% N	-	-	-
LSD 5% K_2O	-	-	-
LSD 5% N x K_2O	-	0.68	-

Notes: DAT = Days After Transplanting, dw = dry weight, ns = non significant, * = significant difference in the level 5%, CV = Coefficient of Variance, LSD = Least Significant Difference

1.6 Stomatal Density

At 90 DAT, observation on stomatal density (mm^{-2}) was only affected by nitrogen application (Table 4). For the average value of potassium treatment, the increasing dosage of nitrogen from 100 into 150 kg N ha^{-1} has not increased the stomatal density per mm^2, but decreased for about 13.71% (from 42.31 into 36.51 mm^{-2}). As well as dosage of nitrogen 200 kg N ha^{-1}, has decreased the stomatal density for about 27.96% (from 42.31 into 30.48 mm^{-2}).

It occurred due to in higher nitrogen application, the cells grow bigger and faster, so that the stomata size became bigger. On the same view range, 0.3545 mm^2, by 400x enlargement using microscope, it brought about higher stomatal density using lower dosage of nitrogen than the higher dosage. This phenomenon has also occurred during the research on nitrogen application to *Jatropha curcas* L., in which the application of 50 kg N ha^{-1} would result higher stomatal density in comparison with the application of 200 kg N ha^{-1} [6].

Table 4. The Effect of Nitrogen (N) and Potassium (K_2O) on the Average of Stomatal Density (mm^{-2})

Treatment (kg ha^{-1})		Stomatal Density
N	K_2O	90 DAT
100	75	40,66
	150	43,01
	225	43,25
150	75	32,44
	150	37,84
	225	39,25
200	75	31,50
	150	35,02
	225	24,91
250	75	37,84
	150	39,02
	225	36,90
N		*
K_2O		ns
N x K_2O		ns
CV (%)		12,93
LSD 5% N		4,65
LSD 5% K_2O		-
LSD 5% N x K_2O		-

Notes: DAT = Days After Transplanting, ns = non significant, * = significant difference in the level 5%, CV = Coefficient of Variance, LSD = Least Significant Difference

1.7 Yield Component

The yield component of stevia during the period I comprised of fresh weight and dry weight, which included leaf, stem, root, and total plant. Interaction between nitrogen and potassium application has significantly increased fresh and dry weight of leaf, stem, and total plant; meanwhile, fresh and dry weight of roots would increase significantly along with each application of nitrogen and potassium (Table 5). Fresh weight of the plant reflected gross yield of photosynthesis due to it still contain water, while dry weight of the plant reflected effectiveness of the plant in absorbing the available resources both within and above the ground.

Availability resources including nutrients have highly affected the formation of biomass, particularly nitrogen. Nitrogen is one of macronutrients, which is highly influential for the plant growth, particularly in formation phase or the vegetative growth, such as root, stem, and leaf. Nitrogen is an essential nutrient to produce dry material of the plant, which regulates photosynthesis and production of the plant (Wu FeiBo et al., 1998 in [8]). On the other side, the role of potassium should not be neglected in the plant growth. When the plant has potassium deficiency, it would not only cause decreasing growth variable, but also change the translocation of photosynthetic product (biomass) among the plant tissues [9].

Fig. 1 describes that the average values of potassium treatment, the increasing dosage of nitrogen from 100 kg N ha^{-1} into 250 kg N ha^{-1} has significantly increased the biomass accumulation in roots, stem, and leaf for about 46.00%, 81.63%, and 140.48%, respectively. If it is related to the nutrient adsorption rate by the plant (Table 7), therefore it would be in harmony. The increasing dosages of nitrogen and potassium are strightly proportional to the additional N accumulation in leaf. This is in accordance with the use of nitrogen as the trigger in vegetative growth as well as the role of nitrogen as macronutrient. Cakmak [10] described that the nitrogen adsorption and its role in plant were also highly supported by potassium, in which the balance between nitrogen and potassium is required. Little potassium content could prevent optimal adsorption of nitrogen and phosphorus application.

Table 5. The Effect of Nitrogen (N) and Potassium (K_2O) on Average Fresh Weight and Dry Weight of the Yield Component (Root, Stem, Leaf, and Total Plant)

Treatment (kg ha^{-1})		Fresh weight (g plant^{-1})				Dry weight (g plant^{-1})			
N	K_2O	Root	Stem	Leaf	Total Plant	Root	Stem	Leaf	Total Plant
100	75	5,70	17,98	3,62	27,30	2,25	3,68	1,14	7,08
	150	8,24	17,73	3,88	29,85	2,80	3,52	0,98	7,31
	225	8,38	21,42	3,95	33,75	2,91	4,28	0,99	8,18
150	75	6,19	22,55	4,51	33,25	2,29	4,52	1,13	7,94
	150	9,68	20,27	5,80	35,74	3,28	3,87	1,43	8,58
	225	11,09	27,03	6,50	44,62	3,81	5,95	1,67	11,43
200	75	7,73	24,01	5,50	37,23	2,64	4,84	1,31	8,79
	150	10,15	24,13	8,42	42,70	3,36	4,97	2,12	10,45
	225	16,25	42,87	12,36	71,48	5,41	8,94	3,11	17,46
250	75	8,15	29,11	7,70	44,96	3,21	6,00	1,81	11,02
	150	9,56	31,96	8,73	50,24	3,29	6,35	2,19	11,83
	225	14,87	41,30	12,84	69,01	5,13	8,51	3,49	17,13
N		*	*	*	*	*	*	*	*
K_2O		*	*	*	*	*	*	*	*
N x K_2O		ns	*	*	*	ns	*	*	*
CV (%)		22,56	14,96	23,87	13,30	23,25	14,22	24,44	13,33
LSD 5% N		2,13	-	-	-	0,76	-	-	-
LSD 5% K_2O		1,85	-	-	-	0,66	-	-	-
LSD 5% N x K_2O		-	6,76	2,82	9,76	-	1,31	0,74	2,39

Notes: DAT = Days After Transplanting, ns = non significant, * = significant difference in level 5%, CV = Coefficient of Variance, LSD = Least Significant Difference

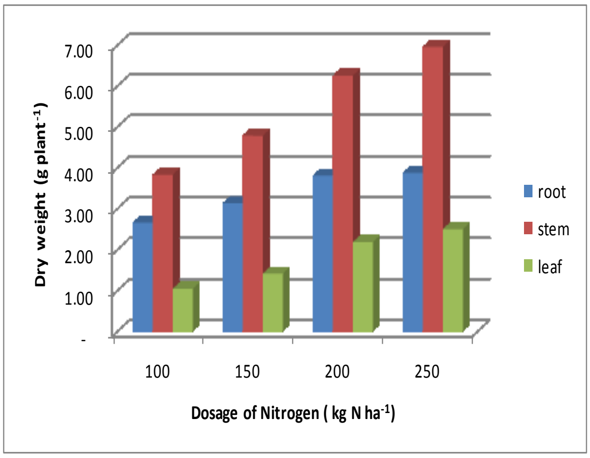

Fig. 1: Effect of nitrogen application on biomass

1.8 Yield

Both fresh and dry weights of leaves per hectare during the period of harvest I, II, and total have shown significant interaction between the applications of nitrogen and potassium (Table 6). The yield of the harvested fresh and dry leaves of stevia that resulted from combination of nitrogen and potassium treatments during the period of harvest II has shown the increasing yield in comparison with the harvest I. Such increasing occurred due to at the first period of harvest, the plants have newly grown and tried to utililize the environmental condition. At the second period of harvest, the plants have kept growing from the ratoon of the previous harvest. Moreover, it had along with different climatic condition between the period of harvest I and II. For condition during the period of harvest II, light intensity was higher (122.9 watt m^{-2}) in comparison with the harvest I (91.6 watt m^{-2}) (data was not presented), so that the plant would absorb much energy from the sun for photosynthetic process and produce more assimilates. The increasing yield of fresh-harvested leaf (kg ha^{-1}) on total harvest as a result of interaction between nitrogen and potassium applications has diverse patterns as presented in Fig. 2.

Table 6. The Effect of Nitrogen (N) and Potassium (K_2O) on Average Fresh Weight and Dry Weight of the Leaf Harvest (kg ha^{-1}) Period of Harvest I, II, and Total

Treatment (kg ha^{-1})		Fresh weight (kg ha^{-1})			Dry weight (kg ha^{-1})		
N	K_2O	Harvest I	Harvest II	Total	Harvest I	Harvest II	Total
100	75	336 a	468 a	804 a	85 a	94 a	179 a
	150	356 ab	581 ab	937 ab	90 ab	123 ab	213 ab
	225	398 ab	518 a	916 ab	89 ab	98 a	187 a
150	75	496 abc	622 ab	1.119 abc	120 ab	119 a	239 ab
	150	531 abcd	770 abc	1.302 abcd	134 abc	158 abc	292 abc
	225	596 bcde	819 abc	1.415 bcde	153 bcde	165 abc	318 bcd
200	75	480 abc	652 ab	1.132 abc	136 abcd	137 ab	272 ab
	150	772 de	1.073 c	1.845 de	199 cde	226 c	426 d
	225	1.133 f	1.647 d	2.780 f	297 f	339 d	636 e
250	75	706 cde	945 bc	1.650 cde	201 de	195 bc	396 cd
	150	800 e	1.120 c	1.919 e	209 e	223 c	432 d
	225	1.177 f	1.740 d	2.917 f	302 f	359 d	661 e
N		*	*	*	*	*	*
K_2O		*	*	*	*	*	*
N x K_2O		*	*	*	*	*	*
CV (%)		23,01	24,24	20,66	23,33	23,63	19,14
LSD 5% N		-	-	-	-	-	-
LSD 5% K_2O		-	-	-	-	-	-
LSD 5% N x K_2O		253	375	546	66	75	115

Notes: values followed by the same letter in the same column have insignificant difference on LSD test 5%, DAT = Days After Transplanting, * = significant difference in level 5%, CV = Coefficient of Variance, LSD = Least Significant Difference, Harvest I = plant at 90 DAT, Harvest II = ratoon at 75 days after Harvest I

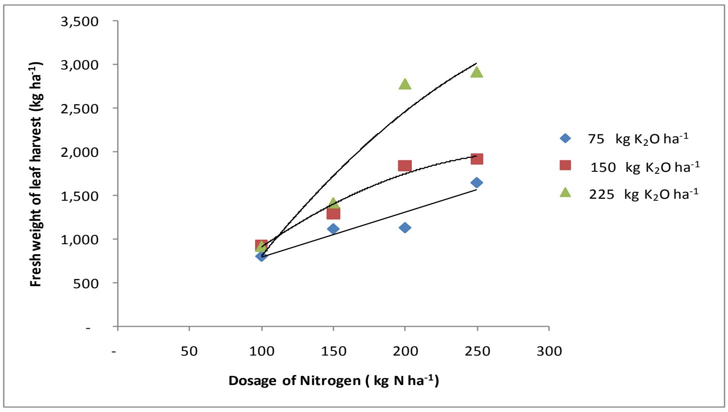

Fig.2: Interaction between N and K on fresh weight of leaf harvest in stevia (kg ha^{-1})

Fig. 2 describes that the fertilization of 100 and 150 kg N ha^{-1}, between the application of potassium in 75, 150 and 225 K_2O ha^{-1} dosages, have not given significant increasing on total fresh weight of the harvested stevia's leaf. At the application of 200 kg N ha^{-1}, the increasing application of potassium from 75 kg K_2O ha^{-1} into 150 kg K_2O ha^{-1} has significantly increased fresh weight of total harvest of stevia's leaf for about 62.99% (from 1,132 into 1,845 kg ha^{-1}). As well as the application of 225 kg K_2O ha^{-1}, this has significantly increased fresh weight of total harvest of stevia's leaf for about 145.58% (from 1.132 into 2,780 kg ha^{-1}). However, the application of 250 kg N ha^{-1}, the increasing application of potassium from 75 kg K_2O ha^{-1} into 150 kg K_2O ha^{-1} has not significantly increased fresh weight of total harvest of stevia's leaf, but when dosage of potassium was increased 225 kg K_2O ha^{-1}, it has significantly increased fresh weight of total harvest of stevia's leaf for about 76.79% (from 1,650 into 2,917 kg ha^{-1}). However, if it is connected with data in Table 6, the application dosage combination of 200 kg N ha^{-1} and 225 kg K_2O ha^{-1} is preferred in stevia. The role of potassium in optimizing nitrogen adsorption by the plant is very high in increasing the yield. This phenomenon has also occurred during the research on application of nitrogen and potassium on barley plant in hydroponic system. At dosage of 50-100 ppm nitrogen, the increasing dosage of potassium from 10 ppm into 50 and 200 ppm has significantly increased the seed yield per plant [11].

1.9 Yield Quality (Stevioside Content)

Stevioside is one of sweetener compounds, which contain in stevia's leaf. Quality of the stevia's leaf is assessed from the content level of steviol glycoside, in which one of them contains stevioside. Stevioside would decrease if any essential nutrient deficiency occurred (Utumi et al., 1999 in [4]). For the average values of potassium, mean level of stevioside in stevia's leaf has increased along with the increasing nitrogen application. The mathematic correlation for the effect of increasing nitrogen dosage on mean level of stevioside is formulated in linear equation of $Y = 0.000408 X + 0.439$ by $R^2 = 0.90^*$ (Fig. 3).

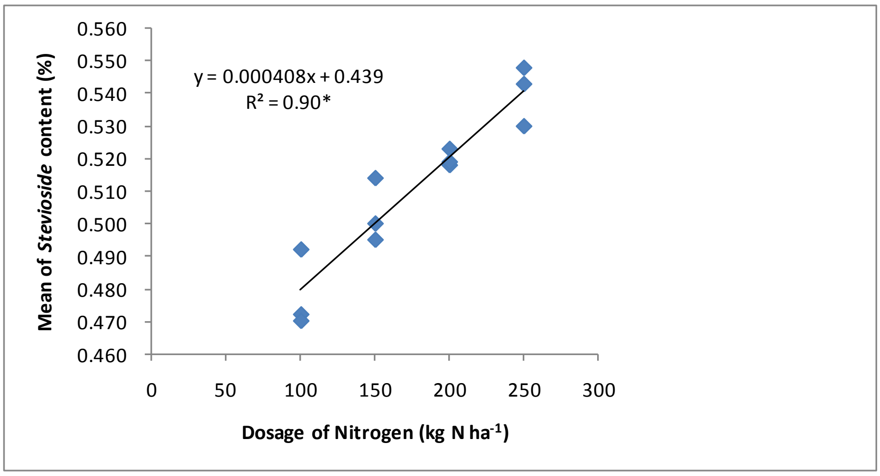

Fig. 3: Relationship between the application of nitrogen and stevioside content (%)

The regression analysis of variance has shown conformity of the simple linear regression model. Based on t-test 5%, the regression coefficient value means that each increasing of one kg nitrogen would increase stevioside level for about 0.000408 to the dosage limit of 250 kg N ha^{-1}.

Research conducted in Egypt described that the increasing dosage of nitrogen from 10 into 30 kg N has significantly increased stevioside for about 1.99 % (Allam et al., 2001 in [4]). Stevioside, which is contained in steviol glycoside, is synthesized in the plant's tissues that contain chlorophyll. Chloroplast as the precursor's site plays its role in synthesizing steviol glycoside (Pol et al., 2007 in [12]). Moreover, Yadav et al. [13] stated that the precursor compounds, which are synthesized in chloroplast, play their roles in synthesizing glycoside, therefore more glycoside accumulate in the mature leaf than the young, and it creates sweeter taste. It is due to in mature leaf, most of the chlorophyll in chloroplast plays their roles in glycoside synthesis. Based on the description above, nitrogen as compositor element of protein, enzyme, coenzyme, nucleic acid, phytochrome, and chlorophyll plays important role in biochemical process of the plant as required by stevia in order to increase stevioside.

1.10 Adsorption of N, P, K in Leaf

Nutrient adsorption is directly related to nutrient content in the plant as well as the biomass yield. Nutrient supplies that conformed to the plant's needs during growth and development phase would produce high biomass (Fig. 1). High nutrient content in plant is related to nutrient availability that can be absorbed by plant in rooting zone [4].

Mean results of N, P and K adsorption have increased along with the increasing dosages of nitrogen and potassium (Table 7). At the average values of potassium treatment, the increasing dosage of nitrogen from 100 kg N ha^{-1} into 250 kg N ha^{-1} has increased N adsorption for about 116.36% (from 385 into 833 kg ha^{-1}), P adsorption 123.53% (from 17 into 38 kg ha^{-1}), and K adsorption 155.20% (from 221 into 564 kg ha^{-1}). Regarding of the adsorption value of the absorbed element, N and K are highly and mostly absorbed by the plant, while P is relatively less absorbed by the plant.

Research by Aladakatti [4] has also shown the same results. On the average value of potassium treatment, the increasing dosage of nitrogen from 200 kg N ha^{-1} into 400 kg N ha^{-1} has increased N adsorption for about 32.55% (from 184 into 243.9 kg ha^{-1}), P adsorption for about 26.60% (from 20.83 into 26.37 kg ha^{-1}) and K adsorption for about 32.70% (from 243.8 into 323.5 kg ha^{-1}). According to Well and Meredith (1984, in [14]) that high concentration of nitrogen in leaf tissues is caused by continuous supply of K^+ through the available K^+ in the soil and the plant as a set of units, which could increase dry weight of the plant. Moreover, as stated by Mengel et al. (1976, in [14]), high adsorption of nitrogen and potassium by the plant due to high potassium application.

Table 7. Nutrient adsorption of N, P, K (kg ha^{-1}) by the Stevia's leaf during the period of Harvest II

Application of N (kg N ha^{-1})	Application of K (kg K_2O ha^{-1})			
	75	150	225	Mean
		N adsorption		
100	442	418	295	385
150	380	663	677	573
200	533	928	983	815
250	469	736	1.294	833
Mean	456	686	812	
		P adsorption		
100	16	19	15	17
150	12	28	22	21
200	19	32	46	32
250	30	34	49	38
Mean	19	28	33	
		K adsorption		
100	187	255	221	221
150	306	380	452	379
200	318	432	900	550
250	357	500	835	564
Mean	292	392	602	

1.11 Chemical Properties of Soil after Harvest II

By the end of experiment, some chemical properties of the soil have changed in comparison with the previous condition before planting. The remained nutrient, N, in the soil has reduced for about 28.13% (from 0.32 into 0.23%) and K for about 72.30% (from 1.48 into 0.41 me), however, the increasing remained-nutrient has occurred on P_2O_5 for about 59.70% (from 26.3 into 42 ppm). In general, it is assumed that such changes are related to status of the previous nutrient and its adsorption by the plant. For N and K element, the adsorption of N and K by the leaf is very high, so that it could reduce the remained N and K in the soil, while for P element, it is assumed that the organic material, such as chicken manure that contains high P_2O_5 (0.82%), which is applied before planting, has been able to provide much nutrients, P_2O_5 , in the soil and on the other side, P adsorption by the leaf has relatively less.

IV. Conclusion

1. Significant interaction between the application of nitrogen and potassium has occurred in parameter of growth, such as number of leaf, leaf area, leaf area index, and chlorophyll level. Separate application of nitrogen and potassium has significantly affected on the plant height, meanwhile the stomatal density is only affected by nitrogen application.
2. Significant interaction between the application of nitrogen and potassium occurred on biomass and the harvest yield of leaf during the period of harvest I, II, and total. At the period of total harvest, the application of 200 kg N ha^{-1}and 225 kg K_2O ha^{-1} has resulted fresh leaf and dry leaf for about 2,780 kg ha^{-1} and 636 kg ha^{-1}, respectively.
3. The increasing application of nitrogen from 100 into 250 kg N ha^{-1} has significantly increased stevioside level in stevia's leaf linearly by the equation of $Y = 0.000408 X + 0.439$.
4. The increasing application of nitrogen and potassium could increase the nutrients adsorption by the plant, such as N, P and K.

References

[1] Hardjowigeno, S., Soil science (Akademika Pressindo, 7th copies, Jakarta, 2010).
[2] Maheshwar, H. M., Effect of different levels of nitrogen and dates of planting on growth and yield of Stevia(Stevia rebaudiana Bert.), magister thesis., Univof Agric Sci, Dept of Hort, Dharwad, 2005.
[3] Aladakatti, Y. R., Y. B. Palled., M. B. Chetti., S. I. Halikatti., S. C. Alagundagi., P. L. Patil., V. C. Patil. and A. D. Janawade, Effect of nitrogen, phosphorus and potassium levels on growth and yield of stevia (Stevia rebaudiana Bertoni). Karnataka. J. Agric. Sci., 25(1), 2012, 25-29
[4] Aladakatti, Y. R.,. Response of Stevia (Stevia rebaudiana Bertoni.) to irrigation schedule, planting geometry and nutrient levels, doctoral diss., Univ of Agric Sci, Dept of Agron, Dharwad, 2011.
[5] Gardner, F. P., R. B. Pearce. and R. L. Mitchell, Fisiologi tanaman budidaya. Herawati Susilo, translator. (UI-Press, Jakarta, 2008, Translation from: Physiology of Crop Plants).
[6] Widaryanto, E. A comprehensive review on Jatropha curcas L. in order to increase yield and benefits, doctoral diss., Univ of Brawijaya, Postgraduate Program, Faculty of Agric, Malang, 2008.
[7] Lei Ma and Yan Shi, Effect of potassium fertilizer on physiological and biochemical index of Stevia rebaudiana Bertoni. Energy Procedia 5, 2011, 581-586.
[8] Sawan, Z. M., S. A. Hafez. and A. E. Basyony, Effect of nitrogen fertilization and foliar application of plant growth retardant and zinc on cottonseed, protein and oil yields and oil properties of cotton. J. Agron & Crop Sci (186), 2001, 183-191.

[9] Reddy, K. R. and D. Zhao, Interactive effect of elevated CO_2 and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton. Field Crops Res 94, 2005, 201-213.
[10] Cakmak, I., Potassium for better crop production and quality. Editorial. Plant Soil (335), 2010, 1-2.
[11] Anonymous, Improving the efficiency of nitrogen use with potassium. International Potash Institute. e-ifc. 13, 2007, 2-3.
[12] Jana, S. Z., S. Voca., N. Dobricevic., D. Jezek., T. Bosiljkov and M. Brncic, Stevia rebaudiana Bertoni- A review of nutritional and biochemical properties of natural sweetener. Agric. Conspectus Sci. 78(1), 2013, 25-30.
[13] Yadav, A. K., S. Singh., D. Dhyani. and P. S. Ahuja, A review on the improvement of stevia [Stevia rebaudiana (Bertoni)]. Can. J. Plant Sci (91), 2011, 1-27.
[14] Makhdum, M. I., H. Pervez and M. Ashraf,, Dry matter accumulation and partitioning in cotton (Gossypium hirsutum L.) as influenced by potassium fertilization. Bio Fertil Soil (43), 2007, 295-301.