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Yield, Capsaicin Content and Peroxidase Enzyme Activity of Four Chili Cultivars on Three Environments

Mahya Nur Rohmah*, Suyadi Mitrowihardjo, Rudi Hari Murti
Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora no. 1, Bulaksumur, Sleman, Yogyakarta 5528, Indonesia
***Corresponding email: mahya.nurr@mail.ugm.ac.id**

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ABSTRACT

The experiment aimed to determine the effect of different environment (altitude and drought stress) on the growth, yield per plants, capsaisin levels, and peroxidase activities of four chili cultivars. *Capsicum annuum* ('Lado' and 'Gada') and *Capsicum frutescens* ('Pelita' and 'Sona') were grown with standard cultivation in medium land (± 700 m asl, Kaliurang) and low land (112 m asl, Bulaksumur) in two environments i.e. standard cultivation and drought treatment. The cultivars were arranged in Complete Randomize Design with four replications in each environment. The data were analyzed using analysis of variance with orthogonal contrast model, while the GGE biplot was applied for identification of stability. The result showed significant interaction between environment and cultivar on plant height, fruit's weight per plant and the contents of capsaicin. Medium land of Kaliurang had suitable environment for chili cultivation of four cultivars and the high capsaicin content of *C. frutescens.* 'Gada' was significantly higher than other cultivars for fruit weight per plant in all planting conditions, while 'Sona' had the highest capsaicin content and was stable in all environments. Drought stress increased capsaicin content in low capsaicin content variety, in contrast to high capsaicin content cultivar. Peroxidase enzyme activity of *C. frutescens* was significantly higher than *C. annuum*, while *C. annuum* cultivars also shown significantly different in peroxidase activity.

Keywords: Capsaicin Levels, Drought Stress, Environment, Enzyme Peroxidase Activity

INTRODUCTION

The genus of *Capsicum* has a high economic value, due to their use as both vegetable and spice. In addition to their importance as a food, chili also have important role in the pharmaceutical or medical field, particularly because of the capsaisinoid content on fruit. Capsaisinoid has been used for the treatment of pain and inflammation associated with various diseases such as rheumatoid arthritis, diabetic neuropathy, postmasectomy syndrome pain, cluster headaches, herpes zoster, and others (Lim, 2013; Srivastava, 2013).

Currently more than 15 capsaicinoids have been found and dominated by capsaicin (Srivastava, 2013; Sung *et al*., 2005), about 48.6% (Kosuge and Furuta, 1970). In most species of Capsicum, accumulation of capsaicin in the fruit begins about 20 days post-anthesis (DPA). Capsaicin biosynthesis occurs in the epidermal cells of the placenta, where they subsequently secreted towards the outer wall of the cell, and eventually accumulated in the structure called blister located on the surface of the placenta (Mazourek *et al*., 2009).

There are several major enzymes involved in the metabolism of capsaicin, one of them is peroxidase. Most of peroxidase activities occur in the placenta and the outer layer of the pericarp epidermis cells. Peroxidase activity increased when capsaisinoid concentration decreased. Measurement of the enzyme activity could be an important indication for capsaisinoid metabolism regulation (Diaz *et al*., 2004; Mazourek *et al*., 2009; Gonzalez *et al*., 2011). Peroxidase does not only play a role in plant defense against bio-tropic and necro-tropic pathogens, but also acts as a biomarker of biotic and abiotic stresses, for example to drought stress. Peroxidase has been strongly suspected to have a role in the metabolism of capsaicin (Diaz *et al.*, 2004).

Planting environment including soil, temperature, and light intensity affect the content of capsaicin (Zamudio-Moreno *et al.*, 2014). Productivity of flowers and chilies tend to decline with drought stress, but on the other hand increase the levels of spiciness of chilies. The response of plants to drought stress depends on the amount of water lost, the level of damage, and the length of drought, also influenced by the plant genotype.

Drougth stress significantly increased capsaicin content on Zest Beauty variety (Sung *et al.*, 2005) and Habanero variety (Ruiz-Lau *et al.*, 2011), but not significant in Hungariana and Home Flavor varieties. It indicates the different response of genotypes. Chili is grown in low land to upland with various environment. There is a lack information of the response of different species in different environments of altitude and drougth stress. This study was aimed to determine the effect of different environments (altitude and drougth stress) and varieties of *Capsicum annuum* and *Capsicum frutescens* (that commonly used by farmer in Indonesia) on growth and productivity of plants, content of capsaicin and peroxidase activity. In this paper exposed the responses of two group genotype to different altitude and drougt condition.

MATERIALS AND METHODS

Experiment was carried out in Seed Development Center for Food and Horticultural Crops, Kaliurang (±700 m above sea level, 29-32^{o}C, normal watering) and Bulaksumur normal (±112 m above sea level, 32-34^{o}C, with normal watering and drought stress treatment). Two cultivars in each species of *Capsicum annuum* ('Gada' and 'Lado') and *Capsicum frutescens* ('Pelita' and 'Sona') were used. The normal watering was applied in medium land of Kaliurang and Bulaksumur while drought treatment applied only in Bulaksumur. In normal watering treatment the plants watered everyday, while in the drought treatment the plants were watered only when the leaves were wilting about once in four days. The cultivars were arranged in Complete Randomize Design with four replications in each environment.

Planting medium used was a mixture of soil and compost in the ratio 1:1. Drought treatment was applied after flowering date. The plant height, fruit weight per plant, capsaicin content, and peroxidase activity were recorded.

Determination of contents of capsaicin

Capsaicin concentration was measured by TLC (Thin Layer Chromatography). The sample was prepared using Matreska and Perucka (2005) method. Sample was measured at 228 nm wavelength and Rf.061. Standard regression curve of capsaicin was 0.0100 g/10 ml FP2x with the function y = 54124x - 1892.5 and R^2 = 0.9995 for estimating the contents of capsaicin.

Peroxidase assay

Sample was prepared using Kar and Dinabandhu (1976) method. The supernatant (as a sample) was inserted into a spectrophotometer cuvette and measured at 420 nm wavelength.

Data analysis

Data were analyzed using analysis of variance in orthogonal contrast model by SAS 9.3, continued by orthogonal contras and GGE biplot (Yan, 2002).

RESULT AND DISCUSSION

The results of orthogonals contrast showed no significant difference in plant height of *C. annuum* and *C. frutescens* in all environments. The results of this study (Table 1) showed the height of *C. annuum* plants ranged from 25.75-92.50 cm, while *C. frutescens* was between 37.38-111.00 cm. This was similar with Lim (2013) that plant height of *C. annuum*

Table 1. Plant height (cm) of four chilli cultivars on three environments in 120 days after planting (dap)

Cultivars	Environments			Average
	112 m asl, normal watering	112 m asl drought treatment	700 m asl, normal watering	
'Gada' (*C. annuum*)	39.75 B c	45.69 H i	64.00 N p	49.81
'Lado' (*C. annuum*)	25.75 B d	28.06 H j	92.50 N o	48.77
'Pelita' (*C. frutescens*)	53.75 A e	63.38 G k	70.75 M r	62.63
'Sona' (*C. frutescens*)	37.38 A f	58.25 G k	111.00 M q	68.88
Average	39.16	48.84	84.56	(+)

Remarks: (+) indicates an interaction between environment and cultivar. The number followed by different capital letters at the same column indicates a significant difference between *C. annuum* vs. *C. frutescens*, while the lowercase letters difference at the same column showed significant difference between cultivar in each genus based on orthogonal contrast test (α = 5%).

Table 2. Weight of fruit per plant (g) of four chili cultivar (100 days after planting) in three planting environments

Cultivars	Environments: 112 m asl, normal watering	112 m asl, drought treatment	700 m asl, normal watering	Average
'Gada' (*C. annuum*)	66.48 A b	19.60 G h	117.16 M o	67.74
'Lado' (*C. annuum*)	5.78 A c	4.08 G h	116.15 M o	42.00
'Pelita' (*C. frutescens*)	33.04 A d	19.67 G i	70.90 N p	41.20
'Sona' (*C. frutescens*)	20.49 A d	12.67 G i	50.99 N p	28.05
Average	31.44	14.00	88.80	(+)

Remarks: (+) indicates an interaction between environment and cultivar. The number followed by different capital letters at the same column indicates a significant difference between *C. annuum* vs. *C. frutescens*, while the lowercase letters difference at the same column showed significant difference between cultivar in each genus based on orthogonal contrast test (α = 5%).

species ranged from 30-80 cm, while the *C. frutescens* between 30-100 cm.

Almost all plant height of different cultivar from the same species showed significant differences with differ order depending on planting environment. 'Gada' and 'Lado' was significantly different in all planting environments. 'Gada' was higher than 'Lado' in normal and drought planting environments in Bulaksumur. However, the reverse result was occurred in Kaliurang. The similar response occurred in 'Pelita' and 'Sona'. The different response of cutivar when grown in different environments indicate the presence of genotype and environment interaction (G x E). Large effects from GXE interaction can directly reduce the contribution of genetic component on plant phenotype. Mattjik *et al.* (2011) stated that the interaction of genotype and environment is very complex because of the variations in environmental components.

The difference of plant height between the plants grown in Kaliurang and Bulaksumur was caused by the planting conditions, such as altitude, temperature, and light intensity. All cultivars were more optimal when grown in Kaliurang which had elevation of 700 m above sea level and light intensity of 750 lux, when compared to the planting conditions in Bulaksumur with an altitude of 112 m above sea level and light intensity of 450 lux. According to Lim (2013), the pepper plants could grow well in all altitude, thus mean that the most influential factor is the intensity of light. It was similar to research done by Sumarni and Agus (2005) who stated that pepper plants required high light intensity so they could have optimal flowering.

Fruit weight of *C. annuum* and *C. frutescens* on normal watering and drougth in low land (Bulaksumur) was not significantly different, but it was significantly different between *C.annuum* and *C. frustecens* in Kaliurang (Table 2). Fruit weights of both species which were produced in Kaliurang were higher than in Bulaksumur, and the response of *C. annuum* was much higher than *C. frustescens*. It indicated that Kaliurang was a better environment for chilli production. The lower ambien temperature, high light intensity, and sandy soil type in Kaliurang could promote the optimum photosynthesis and impacted on higher growth and yield of Capsicum. This was similar to a statement from Lim (2013), that *C. annuum* and *C. frutescens* were suitable to be planted in sandy soils.

Table 2 showed the interaction between genotype and environment. Interaction of genotype x environment could be visualized in the polygon patterns on graph biplot "the which-won-where". Genotyping in a point angle polygons for each sector was a genotype that produces the largest fruit weight per plant on the environment (L) which exist at the same sector (Yan, 2002). 'Gada' variety produced highest fruit weight per plant in all environments (Figure 1A), while 'Sona' variety was the lowest in all envonments indicated by oposite position from all environments. This result could predict that 'Gada' was the higest and most stable on fruit weight variable as shown in the average environement coordinate method.

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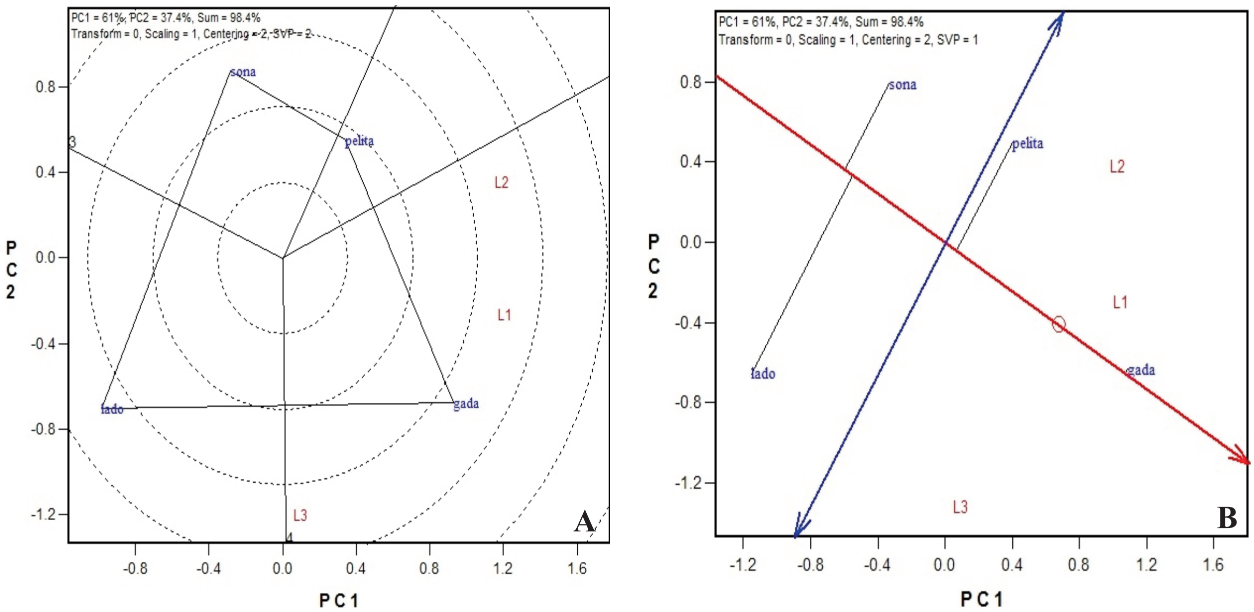

Figure 1. (A) GGE biplot (symmetrical basis scale with pattern of which-won-where) of fruit weight per plant; (B) GGE biplot (symmetrical basis scale with coordinate pattern of average environment) for fruit weight character per plant; L1= Bulaksumur normal, L2= Bulaksumur with drought stress, L3= Kaliurang.

Table 3. Capsaicin level (mg/g) of four chili cultivar (80 days after flowering) on three environments

Cultivars	Environments			Average
	112 m asl, normal watering	112 m asl, drought treatment	700 m asl, normal watering	
'Gada' (*C. annuum*)	15.39 B d	25.55 G h	23.81 N o	21.58
'Lado' (*C. annuum*)	156.72 B c	98.36 G h	46.31 N o	10.73
'Pelita' (*C. frutescens*)	123.90 A e	58.24 G j	166.12 M q	11.09
'Sona' (*C. frutescens*)	184.86 A e	158.72 G i	253.18 M p	19.92
Average	120.22	84.02	122.35	(+)

Remarks: (+) indicates an interaction between environment and cultivar. The number followed by different capital letters at the same column indicates a significant difference between *C. annuum* vs. *C. frutescens*, while the lowercase letters difference at the same column showed significant difference between cultivar in each genus based on orthogonal contrast test (α = 5%).

indicated by oposite position from all environments. This result could predict that 'Gada' was the higest and most stable on fruit weight variable as shown in the average environement coordinate method.

Genotypic stability could be evaluated using average environment coordinate method (Yan, 2002). Average environment was defined as the average score of PC1 and PC2 for all environments, depicted by the small circle on graph GGE biplot (Figure 1B). High mean yield, coupled with high stability in all environment, was the expected cultivar. As depicted in figure 1B, the single-arrowed line called average-environment coordination abscissa points to higher mean fruit weight across the environment. 'Gada' was in the highest abscissa point and with low variance so 'Gada' was the most stable cultivar in all locations (Figure 1B).

The response of varieties in environment variations was different as indicate of significant interaction (+) in Table 3. Contents of capsaicin *C. annuum* was significantly lower than *C. frutescens* in Bulaksumur with normal watering and Kaliurang. It was in accordance with the result from Sung *et al*. (2005) that capsaicin level of *C. annuum* was lower than *C. frutescens*. Musfiroh *et al*. (2013) also showed levels of capsaicin from 9 samples of *C. annuum* (cultivar 'Grossum' and 'Tanjung') were lower than 3 samples of *C. frutescens* (the name of the cultivar was not mentioned).

Capsaicin content of 'Gada' (15.39 mg/g) and 'Lado' (156.72 mg/g) were significantly different at Bulaksumur normal environment. This finding was consistent with the result in Gonzalez-Zamora (2013), that the variations of capsaicin in 8 cultivars *C. annuum* were large (from 0.17 to 15.36 mg/g). It

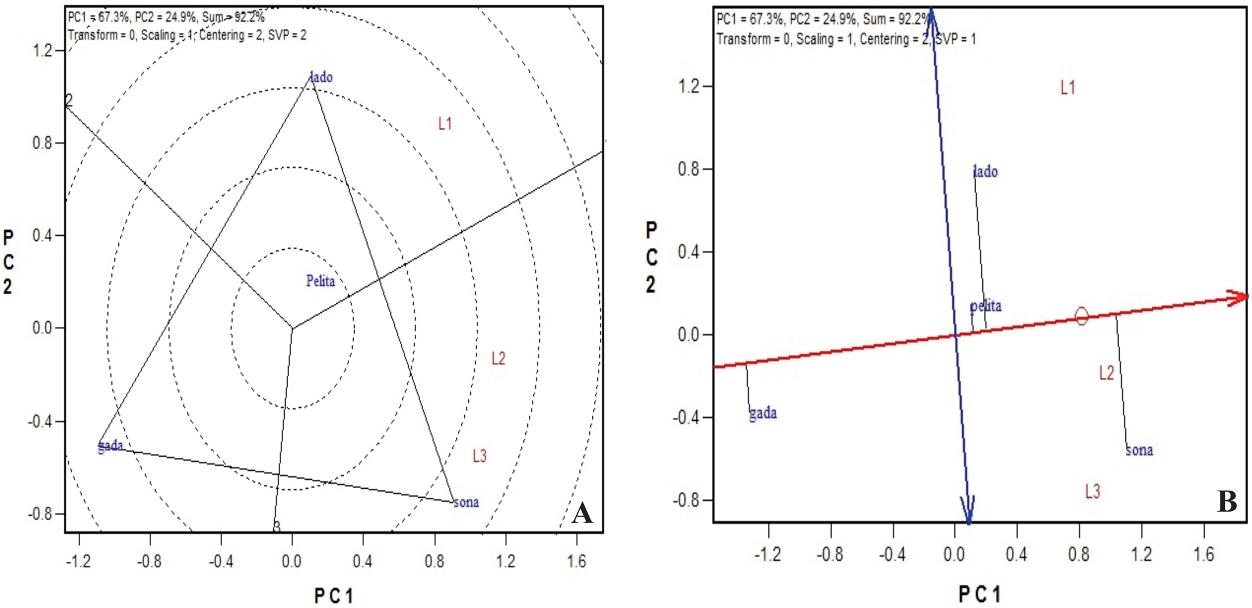

Figure 2. GGE biplot (base symmetrical scale graphic with which-won-where pattern) of capsaisin level (A); GGE biplot (base symmetrical scale graphic with coordinate pattern in average environment) of capsaisin level (B); L1= Bulaksumur normal, L2= Bulaksumur with drought stress, L3= Kaliurang.

Table 4. Rate of peroxidase enzyme (Δ420/minute/mg protein) in four cultivar of chili (80 days after flowering) at three environments

Cultivars	Environments: 112 m asl, normal watering	112 m asl, drought treatment	700 m asl, normal watering	Average	
'Gada' (*C. annuum*)	0.42	0.47	0.66	0.52 d$^{2)}$	0.78 b$^{1)}$
'Lado' (*C. annuum*)	1.78	0.90	0.45	1.04 c$^{2)}$	
'Pelita' (*C. frutescens*)	1.48	1.01	1.49	1.33 e$^{3)}$	1.21 a$^{1)}$
'Sona' (*C. frutescens*)	1.25	1.00	1.01	1.09 e$^{3)}$	
Average	1.23 q$^{5)}$	0.85 q$^{5)}$		(-)	
	1.04 p$^{4)}$		0.91 p$^{4)}$		

Remarks: (-) indicates no interaction between location and cultivar; *C. annuum* (Gada and Lado), *C. frutescens* (Pelita and Sona); a number followed by a different letter on each column or row indicate a significant difference test based on contrast orthogonals 1) *C. annuum* vs *C. frutescens*; 2) Gada vs Lado; 3) Pelita vs Sona; 4) Bulaksumur vs Kaliurang; 5) Bulaksumur normal vs drought conditions (α = 5%).

showed that capsaicin content variation within the same species was very high. In fact, in this study, the level of capsaicin in 'Lado' was too high, compared to the range of capsaicin stated by Gonzales and Zamora. On the other hand, although in general the levels of capsaicin on the cultivar of *C. annuum* were lower compared to *C. frutescens*, the chances of developing *C. annuum* cultivar with high levels of capsaicin is still possible by exploring the levels of capsaicin variation on the other cultivar of *C. annuum*.

Capsaicin levels of 'Pelita' and 'Sona' in Bulaksumur normal conditions were not significantly different. This showed that levels of capsaicin in both cultivar had not yet reached its optimum level at Bulaksumur normal conditions. It was proven by capsaicin levels on Kaliurang planting condition, where the two cultivars were much higher and significantly different when compared to Bulaksumur, where the levels of capsaicin of 'Sona' was also higher than 'Pelita'. The result was similar with Sung *et al*. (2005), that differences in levels of capsaicin were not only determined by chili species differences, but also caused by the difference in the level of cultivar or cultivars, and greatly influenced by its environmental conditions.

Capsaicin contents of 'Gada' and 'Lado' in Kaliurang planting condition were not significantly different and not too high, compared with the two other environments. Although the character of growth and yield of 'Sona' and 'Pelita' were more optimal in this planting condition as discussed previously. This showed the GXE interactions capsaicin contents in these two cultivars. Zamudio-Moreno *et al*. (2014)

stated that the levels of capsaicin strongly influenced by environmental conditions and also by genotype and environment interaction.

Four contents of capsaicin in the chili cultivar at drought Bulaksumur were not higher than in normal Bulaksumur and Kaliurang. This was contrast with the results by Bosland and Votava (2000) and Sung *et al*. (2004) which revealed that the drought increased the contents of capsaicin as a result of oxidation of capsaicin. Capsaicin contents were not increased by the possibility of duration of influenced by drought stress (fewer or more than four days) and level (moisture) of drought given. Sung *et al*. (2005) state that the response of plants to drought stress depends on the amount of water lost, the level of damage and the length of drought.

GGE biplot graph (Figure 2A) of capsaicin content showed 'Lado' variety had high content of capsaicin when grown in normal watering in Bulaksumur. In contrast, 'Sona' variety had a high content of capsaicin when grown in Bulaksumur with drougth stress and Kaliurang.

The performance and stability of the genotype results could be evaluated with the average environment coordinate method (Yan, 2002). Average environment was defined as the average score of PC1 and PC2 in all environments, depicted by the small circle on graph GGE biplot (Figure 2B). Straight line passing through the average environment coordinates of the point of origin means biplot environment called axis means the environment that acts as axis (the tip of the arrow). GGE chart patterns mean environmental coordinate indicated that cultivar with stable levels of capsaicin in two locations and with drought stress was 'Pelita' cultivar (Figure 2B) but the mean was lower than 'Sona'. Capsaicin content of 'Sona' was responsive to environments so it could be explored with growing 'Sona' in a suitable environment such as Kaliurang.

Peroxidase Enzyme Activity and Its Relation to Capsaicin Level

There was a significantly difference in peroxidase activity based on species differences. Peroxidase enzyme activity in *C. frutescens* was higher than in *C. annuum* (Table 4). The peroxidase has been strongly suspected having a role in the metabolism of capsaicin. This enzyme was able to oxidize capsaicin into 5,5'-dicapsaisin and 4'-O-5-dicapsaisin with the presence of H_2O_2. Thus, the high activity of peroxidase enzyme in *C. frutescens* could be related to its ability to oxidize the levels of capsaicin.

Peroxidase enzyme activity differences between the two varieties were likely related to its function in the contents of oxidized capsaicin. The significant difference of peroxidase was also shown between 'Gada' and 'Lado'. 'Lado' capsaicin contents were higher than 'Gada', as shown in the preceding discussion. Correlation analysis of capsaicin and peroxidase contents in three environments showed significant correlation ($r = 0.39$, $p = 0.02$). This result was contrast with the results from Contreras-Padilla and Yahia (1998) which found that the correlation between the contents of capsaicin and peroxidase was a negative. This was likely indicated that oxidation activity had not been performed at the age of 80 days after flowering in four varieties, because the contents of capsaicin had not yet reached its peak before starting to decline.

Diaz *et al*. (2004) stated that the peroxidase activity increased when the concentration of capsaicin decreases (80 days after flowering), so that the negative correlation between the contents of capsaicin and peroxidase enzymes only occured when the contents of capsaicin reaches the highest contents. Contreras-Padilla and Yahia (1998) also emphasized that the role of the oxidation of the peroxidase reasonably believed to be in the final phase of development of the fruit followed by loss of spiciness. Contrary to these results, at the age of 80 days after flowering there was no negative interaction between the enzyme peroxidase with higher contents of capsaicin. The effect of different varieties may also affect to the time when peroxidase begins to oxidize capsaicin.

This condition could also be explained by Sung *et al*., (2004) and Zamudio-Moreno *et al*., (2014) who stated that the oxidation of capsaicin does not rely solely in peroxidase activity, so that other catabolic pathway might also play a role.

CONCLUSION

Kaliurang has a suitable condition for cultivating four cultivar of chili. Fruit weight per plant of 'Gada' cultivar was the highest in all planting conditions. Capsaicin level of 'Pelita' was the most stable, while 'Sona' was the most responsive to environment. Drought stress was not always induced the increasing levels of capsaicin. Enzyme peroxidase activity of *C. frutescens* was significantly higher than *C. annuum*, while among the cultivars in *C. annuum* also showed significantly difference.

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