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Effect of GA_{4+7} treatment on the capsaicinoid content of chilli (*Capsicum annuum* L. var. Kulai)

(Kesan rawatan GA_{4+7} terhadap kandungan kapsaisinoid cili (*Capsicum annuum* L. var. Kulai)

I. S. Usman*, A. S. Mamat**, A. W. Ahmad*** and A. R. Anuar****

Key words: capsaicinoids, pungency, plant growth regulator (PGR), GA_{4+7}, flowering, chilli

Abstrak

Kesan rawatan GA_{4+7} terhadap kapsaisinoid cili telah dikaji pada buah cili yang ditanam di dalam rumah kaca dan di ladang. Sebatian kapsaisiniod utama – kapsaisin (CAP), nordihidrokapsaisin (NDHC) dan dihidrokapsaisin (DHC) telah dipisahkan dan dijumlahkan menggunakan HPLC dan pengenalan sebatian ini disahkan menggunakan GCMS. Jumlah kapsaisinoid yang lebih tinggi telah diperoleh dengan rawatan GA_{4+7} berbanding dengan buah cili yang tidak dirawat. Kesan GA_{4+7} yang lebih jelas telah diperhatikan terhadap NDHC dan DHC. Kandungan kapsaisin telah meningkat secara signifikan apabila rawatan GA_{4+7} dilakukan pada masa puncak pembungaan (PF) dan aplikasi kembar di awal pembungaan dan puncak pembungaan (EF + PF) sahaja. Perbezaan antara buah cili yang dirawat di rumah kaca dan ladang tidak signifikan.

Abstract

The effect of GA_{4+7} treatment on the capsaicinoid content of chilli was studied in chilli fruit grown under glasshouse and field conditions. The major capsaicinoids – capsaicin (CAP), nordihyrocapsaicin (NDHC) and dihydrocapsaicin (DHC) were separated and quantified by HPLC and their identity further confirmed by GCMS. Higher total capsaicinoid content was obtained with GA_{4+7} treatment compared with the untreated control. More pronounced effect of GA_{4+7} was observed on NDHC and DHC. The capsaicin content was significantly increased by GA_{4+7} treatment at peak flowering (PF) and with double application at early flowering and peak flowering (EF + PF) only. The difference between glasshouse and field treated fruit was not significant.

Introduction

Chilli fruit has been used for centuries as spices and for their medicinal value. The fruit is widely consumed, but the pattern of consumption varies with localities and individuals. Some prefer it hotter while others prefer a milder taste. The idea behind these uses is associated with its pungency which is due to the presence of capsaicinoids i.e. vanillic amides of isodecylanic acid in the septa and the placenta of the fruit. The pungency of chilli which is genetically

*Audu Bako School of Agriculture, Dambatta, P. M. B. 3401, Kano, Nigeria
**Faculty of Science and Literature, UKT, Mengabang, Talipot 23101, Kuala Terengganu, Malaysia
***Strategic, Environment and Natural Resources Research Centre, MARDI Headquarters, P. O. Box 12301, 50774 Kuala Lumpur, Malaysia
****Soil Science Department, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
Authors' full names: Inuwa Shehu Usman, Awang Soh Mamat, Ahmad Abdul Wahab, Anuar Abdul Rahim
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determined is modified by environmental factors, such as high levels of water, nitrogen in the soil, and low air temperature (Mary and Balakrishnan 1990; Cotter 1977; Heiser 1976). That capsaicinoid formation in chilli fruit could be modified through plant growth regulator treatment has been reported using ethepon (Perucka 1996).

Gibberellin (GA_3) has been shown to influence fruit quality of horticultural crops (Stembridge and Morell 1972; Norman 1979; Duane 1984). In chilli, GA_3 treatment was reported to increase fruit ascorbic acid and citric acid contents. Similarly GA_3 when used in combination with other plant growth regulators such as Biozyme, increased fruit soluble solids, fructose, sucrose carotenoids and lycopene contents (Belkabir et al. 1998). Capsaicinoids as the chemical constituents responsible for pungency in chilli are the quality determinants. Owing to their unique properties, there is a need to explore further the different ways of modifying the pungent principles in chilli. This paper reports on tests carried out to determine the capsaicinoid content of chilli fruit as affected by GA_{4+7} treatment.

Materials and methods

The experiment was carried out under the field and glasshouse conditions between June 1995 and May 1996. The treatments involve the application of GA_{4+7} on chilli variety Kulai at the rate of 10 µg/mL at different growth periods corresponding to early flowering (EF); peak flowering (PF); and double application at early and peak flowering (EF+PF) together with a control (plants treated with water only). The design was a randomised complete block replicated four times. Field plots consisted of eight plants in a row, while the glasshouse plots contained four plants. Three plants were tagged in the net plot for observations.

GA_{4+7} treatments were applied at 7 weeks after transplanting (WAT) for early flowering and at 10 WAT for peak flowering. Control plants were treated with distilled water only. The entire plant surface was sprayed until first drip (approximately 50 mL/plant spray solution). Data collected were subjected to analysis using SAS system. Means were compared using Ryan-Einot-Gibriel-Welsch multiple range test (SAS Institute Inc. 1988).

Cultural operations

Seeds pre-treated with benlate (benomyl, 0.1 kg a.i./ha) were sown in shallow boxes (50 cm x 25 cm x 15 cm) containing a mixture of sand and coconut waste (1:1). Sowing was in drills 5 cm apart at the rate of one seed/2.5 cm. Shade was provided for the initial 3 weeks and there after removed to harden the seedlings. Daily irrigation with a weak nutrient solution (electrical conductivity 1.0 *mhos*) of Cooper formulation (Cooper 1979) was practised from 2 weeks after sowing, before this seedlings were watered with tap water. Five weeks old seedlings were transplanted into polystyrene bags (for glasshouse experiments) or on ridges (for field experiment).

Transplanting in the glasshouse was in polybags (30 cm diameter x 45 cm long) filled with 7 kg of soil mixture containing top soil, sand and chicken dung (3:2:1 v/v). The polybags were spaced 60 cm x 80 cm. Plants were kept well watered using drip irrigation. Ten grams of compound fertilizer (NPK 15:15:15) was applied to each pot 1 WAT and then at 3 weeks interval. Plants were sprayed biweekly with selecron (prefenofos, 0.1 kg a.i./ha) and just before flowering with benlate (benomyl, 0.2 kg a.i./ha) to control pests and diseases. The entire plot was lined with silver coated plastic sheets before plot layout to discourage weeds and minimize disease spread. Wooden stakes were provided for each plant.

The field was prepared by rotovating twice, followed by ridging. A period of four weeks was allowed between rotovation for weeds to decompose. Seedlings were transplanted on ridges that were previously covered with silver coated plastic.

Supplementary irrigation was provided when necessary by using sprinkler irrigation system. Plant spacing, fertilizer application, staking, pest and disease control were as in the glasshouse experiment.

Capsaicin determination

Sample preparation (after Attuquayerfio and Buckle 1987) Mature chilli fruit was harvested at 6 WAT (6 weeks after last GA_{4+7} treatment), dried in the oven (60 °C) for a period of 5–6 days and ground into fine powder. The dehydrated ground chilli (1 g) is agitated with 10 mL of acetonitrile for 2 minutes on a votex mixer (Votex Co., Japan). The extract (1 mL) is diluted with 9 mL of water and passed into a conditioned C_{18} Sep-pak. (Waters Associates Co. USA). This was achieved by passing 5 mL of acetonitrile followed by equilibration with 5 mL of double-distilled water. The capsaicinoids are then eluted with 4 mL of acetonitrile followed by 1 mL of acetonitrile containing 1% acetic acid.

Preparation of standard solutions Standard solutions of capsaicin were obtained from Merck, Germany. Stock solutions of 10 mg/mL of capsaicin were prepared in the extraction solvent (acetonitrile). Dilutions were made using different volumes of water to give serial polarity grading. The standards were introduced in 10 mL portions on to the conditioned Sep-pak and eluted using same solvents as with the unknowns. The effectiveness of the solvent in eluting the capsaicinoids was determined by assaying a further 1 mL of acetonitrile from the Sep-pak for residual capsaicinoids.

High performance liquid chromatography (HPLC) analysis The high performance liquid chromatograph (HPLC) procedure used a Waters Associate (USA) liquid chromatograph equipped with a 600 multisolvent delivery pump, a 474 auto sampler, a Nova-pak C_{18} column (150 cm x 4.9 mm internal diameter, 5 µm pore size) and a 486 UV turnable absorbance detector set at 280 nm and 0.01 aufs. The mobile phase is methanol/water (63:37, v/v) at a flow rate of 1 mL/min. The injection volume was 10 µL and a run time of 20 minutes.

Gas chromatography – masss spectrometry (GC-MS) analysis Three grams of the dehydrated ground chilli is extracted with 30 mL of acetone for 45 minutes in a soxtec (Tecator Co. UK) apparatus. The extract is then made up to 50 mL with acetone, N, 0-*bis*-trimethylsililyl-trifluoroacetamide (BSTFA) (Pierce Chemical Co., Rockford, UK) was used as a silylation reagent. One mL of the acetone extract is diluted with 1 mL of tetrahydrofuran (THF). One half mL of this mixture was transferred into a 2 mL reacti-vials, followed by addition of 50 mL of BSTFA. The vial is then sealed with a rubber septum cap and allowed to stand for 5 minutes. The silylylated mixture (1 µL) was injected into the GC-MS.

The GC-MS used was a Finningan (USA) quadruple gas chromatography – mass spectrometer equipped with SE 30 column (183 cm x 2 mm internal diametre). The initial temperature of the gas chromatograph was 165 °C and this was increased at the rate of 5 °C/min to a final temperature of 245 °C. This temperature was maintained for 30 min. The injection port temperature was 220 °C. The mass spectra was detected in electron ionisation (EI) mode. Masses in the range 50–450 were scanned, using a scan rate of 100 scans/sec.

Results and discussion

Figure 1 shows the HPLC chromatogram separation of major capsaicinoids from standard and samples. A look at the chromatogram for standard showed that NDHC was eluted from the column at about 6.0 min while the retention time (Rt) for CAP and DHC were 6.7 and 10.2 min respectively. Similar Rt values were obtained for the major capsaicinoids in sample runs. This indicated that

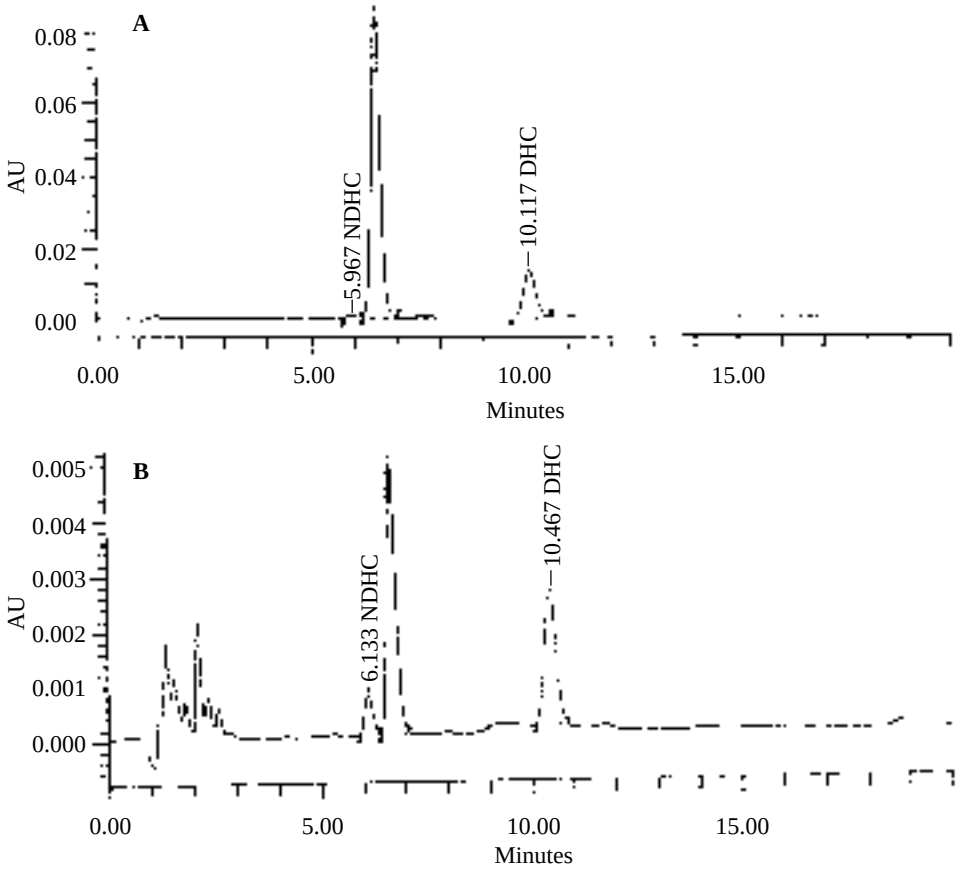

Figure 1: HPLC chromatograms showing the separation of capsaicinoids in (A) standard and (B) typical sample

capsaicinoids were well separated in both standard and sample runs. The chromatograms obtained compare very well to that reported by Attuquayefio and Buckle (1987). For calibration, HPLC runs were made with standard capsaicin of different concentrations. The response was found to be linear up to 500 mg/mL. The capsaicinoids were calculated using individual response factors of 1.0, 1.05 and 1.02 for CAP, NDHC and DHC, respectively (Weaver and Awde 1986; Hoffman et al. 1983).

GC-MS analysis was carried out to confirm the presence of the individual capsaicinoids. According to Ahmad (1984), the trimethyl silyl (TMS) of these components were reported to have parent molecular ion masses of 377, 379, 365, 391 and 393, respectively. They have a common base peak m/z = 209 and other fragment ions of m/z = 73, 179, 194, 224, and 267. CAP, DHC, and NDHC are the major components in most species of capsicum, constituting 95% or more of the total capsaicinoids.

The capsaicinoid content of chilli fruit as influenced by application of GA_{4+7} at various stages of plant growth is shown in *Table 1*. Total content of capsaicinoid was significantly influenced by GA_{4+7} application. Higher total capsaicinoids were obtained with treated plants than with control. Changing the time and frequency of GA_{4+7} application did not have a significant effect on total capsaicinoids.

The content of DHC and NDHC were increased to total capsaicinoid content by

Table 1. Capsaicinoid content of chilli as influenced by GA_{4+7} application at various stages of growth under field and glasshouse conditions

Stage of growth/ Location	TCAP (µg/g)	DHC (µg/g)	CAP (µg/g)	NDHC (µg/g)
Appl. time (T)				
Control	356.2b	106.4a	248.3b	1.6b
EF	700.5a	239.4a	456.4ab	4.7a
PF	763.3a	227.5a	531.5a	4.2a
EF + PF	918.1a	277.2a	635.4a	5.5a
SE (±)	335.47	104.57	231.61	2.42
Location (L)				
Field	758.6a	234.5a	519.6a	4.5a
Glasshouse	610.4a	190.7a	416.1a	3.6a
SE (±)	148.23	43.84	103.49	0.89
T x L Interaction	ns	ns	ns	ns

Means followed by same letter(s) within a group in a column are the same using Ryan–Einot–Gabriel–Welsch Multiple Range Test (p= 0.05).

EF – Early Flowering
PF – Peak flowering
SE – Standard error
ns – not significant.
NDHC – nordihydrocapsaicin
CAP – capsaicin
DHC – dihydrocapsaicin and
TCAP – total capsaicinoids

GA_{4+7} treatment. Significantly higher capsaicin content was obtained with GA_{4+7} at PF and EF + PF, while EF treatment gave capsaicin values that are similar to the control. The difference between glasshouse and field treated fruit was not significant. Similarly, the interaction between location was not significant on the capsaicinoid content of the fruit.

In terms of pungency, sensory heat values were reported to be similar for CAP and DHC (Todd et al. 1977). However, CAP is associated with short term sensory heat effect, while DHC has a higher long term sensory effects. The more pronounced effect of GA_{4+7} on DHC observed in this study tends to indicate that GA_{4+7} can be used to increase the long term sensory effects of chilli. Findings could be of significance in disease management of the crop. Chew (1987) observed a strong relationship between CAP content and tolerance of chilli breeding lines to anthracnose disease and reported that within a particular species the lines that contained higher CAP tend to be more tolerant than those with lower CAP content.

Conclusion

Capsaicinoids, the unique pungency principles in chilli were determined from fruits treated with GA_{4+7}. The contents of nordihyrocapsaicin, capsaicin, dihydrocapsaicin and hence the total capsaicinoids were significantly increased by the GA_{4+7} treatment. Findings could be of significant importance when it is desired to increase capsaicinoids content to meet consumer preferences and improve the anthracnose disease tolerance of the crop.

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References

Ahmad, A. W. (1984). A combined gas chromatography-mass spectrometry (GC-MS) study on the pungent principles in chillies (*Capsicum spp*.). *MARDI Res. Bull*., ***12:*** 290–7

Attuquayefio, K. V. and Buckle K. A. (1987). Rapid sample preparation method for HPLC analysis of capsaicinoids in capsicum fruits and oleoresins. *J. Agric. Food Chem*. ***35:*** 777–9

Belkabir, A., Ruiz, J. M. and Romero, L. (1998). Yield and fruit quality of pepper (*Capsicum annuum* L.) in response to bioregulators. *Hort. Sci*. ***33:*** 85–7

Chew, B. H. (1987). Capsaicin – its' relationship with fruit characters and resistance to anthracnose disease in chillies. *Malays. Appl. Biol.* ***16:*** 269–74

Cooper, A. (1979). *The ABC of NFT* 181 pp. London: Grower Books

Cotter, D. J. (1977). A review of studies on chilli. *Bull. N. M. State Univ. Agric. Exp. Stn.* 673

Duane, W. G. (1984). Microdroplet application of GA_{4+7} + BA: sites of adsorption and effect on fruit set, size and shape of 'Delicious' apples. *J. Amer. Soc. Hort. Sci.* ***109:*** 28–30

Heiser, S. B. (1976). Peppers *Capsicum* (Solanaceae). In: *Evolution of crop plants.* (Simmond ed.) p. 265–8 London: Longman

Hoffman, P. G., Lego, M. C. and Galetto, W. G. (1983). Separation and quantitation of red pepper major heat principles by reverse phase high-pressure liquid chromatography. *J. Agric. Food Chem*., ***31:*** 1326–30

Mary, S. S. and Balakrishnan R. (1990). Effect of irrigation, nitrogen and potassium on pod characters and quality in chilli (*Capsicum annuum* L Cv. K2). *South Indian Hort*., ***38:*** 68–89

Perucka, I., (1996). Effect of 2-chloroethylphosphonic acid on phenylalanine ammonia-lyase activity and formation of capsaicinoids in placenta of hot pepper fruits. *Acta Physiol. Planatarum*, ***18:*** 7–12

SAS Institute Inc. (1988). SAS/STAT* User's guide, Release 6.03 edition. 1028 pp. Cary, NC

Stembridge, G. E. and Morell, G. (1972). Effects of giberellins and 6-benzyladenine on the shape and fruit set of 'Delicious' apples. *J. Amer. Soc. Hort. Sci*. ***97:*** 464–7

Todd, P. H., Bensinger, J. R. and Biftu, T. (1977). Determination of pungency due capsaicin by gas-liquid chromatography. *J. Food Sci*. ***42:*** 660–5

Weaver, K. M. and Awde, D. B. (1986). Rapid high-performance liquid chromatographic method for the determination of very low capsaicin levels. *J. Chrom*., ***367:*** 438–42

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