

Ureide Content of Guar under Influence of Hexaconazole and Triazophos

Yuvraj D Kengar* and Bhimarao J Patil

Department of Botany, Kanya Mahavidyalaya Islampur College, Karad, Maharashtra, India

Abstract

The important part of regular human diet is vegetable, which supply minerals and nutrients for maintenance of health. India is first rank producer of guar comprise 83% of total world production but these are affected by diseases like leaf spots, leaf blotch and insect pest such as serpentine leaf miners, hairy caterpillars and jassids. The major biotic constraint in vegetable production is pest problem. Application of pesticides has now become a common practice in modern agriculture. Chemicalization of agriculture rescues the crop from pest and diseases but when applied in excessive dose affects on plant health and metabolism. The various stresses alter the plant metabolism including pesticidal stress. The ureide content is reported in this study under pesticidal stress of Hexaconazole and Triazophos in leguminous vegetables guar (*Cyamopsis tetragonoloba* (L.) Taub.) after seed treatment and foliar sprays to ensure the nitrogen fixation and metabolism. Changes in the correlation between nitrogen fixation and ureide levels accelerate disturbance in the nitrogen metabolism and nitrogen products in plant. The concentrations of both pesticides for this experiment were 0.05, 0.1, 0.15, 0.2 and 0.3%. The seeds were soaked in these concentrations of pesticides for 12 h. After 12 h seed soaking period, the treated seeds were thoroughly washed with distilled water and sown in earthen pot containing garden soil and manure. The first foliar spray of Hexaconazole and Triazophos were applied with respective concentrations on 10th day while second foliar spray was on 25th day of plant growth. Analysis of ureide was carried out on the 15th and 30th day of growth that is 5 days after each spray. The ureide content in guar was remarkable increased after seed treatment followed by first foliar spray of 0.10, 0.15 and 0.20% hexaconazole however decreased it with dose concentrations of second foliar spray. The ureide content was decreased after both the foliar sprays of Triazophos.

Keywords: Ureide; Guar; Hexaconazole; Triazophos

Introduction

Legumes are agronomical and economical important in many cropping systems because of their nitrogen assimilation ability from atmosphere through nodules, anticipated to increase with it's need in sustainable agricultural practices development. Indeed, biological N₂ fixation is the most significant natural pathway through introduction of nitrogen into the biosphere has been administered. The nodulation on legumes can be determinate or indeterminate development pattern, consisting of three major tissues involving: a central infection zone, an inner cortex that includes vascular bundles, and an outer cortex [1,2]. The carbohydrates and other metabolites are diffusion to the nodule zone via phloem in the nodule cortex and the products of N₂ fixation, either amides (mainly asparagine) or ureides (allantoin and allantoic acid), are exported to the shoot via the xylem [2,3]. Ureide represents the sum of allantoin and allantoate. The point to remobilization of nitrogen from the oldest leaves as the main source for ureide synthesis and accumulation in shoots and developing tissues in the nonnodulated plants, and for the sharp increase in ureides during early pod filling in the nodulated plants when nitrogen fixation starts to decline. Changes in the correlation between nitrogen fixation and ureide levels with transition to reproductive development have been clearly demonstrated [4]. The major accumulation of ureides occurs has completely inhibited the N₂ fixation in nodulated plants [5]. Remobilization of nitrogen from senescent tissues was suggested as the most likely alternative source of ureides and involved in the N-feedback regulation of nitrogen fixation [6]. Moreover, ureide accumulation upon drought stress has been hypothesized to be responsible for N₂ fixation inhibition [7]. The ureide composition under pesticidal stress is not been reported in leguminous vegetables plants to ensure the nitrogen fixation and metabolism altering effect.

Vegetables are essential part of our human regular diet which supply nutrients and minerals for the good health and proper functioning of human body. Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a leguminous

vegetable, having importance due to gum and guran production, utilized in numerous modern manufacturing and food processing industries. Inclusion of vegetables in daily diet is very essential [8]. However, Guar is frequently attacked by diseases and insect pests. Application of pesticides as seed treatments and foliar sprays has become a common practice in modern agriculture [9], although application of pesticides interfere biochemical process of plants [10]. Hexaconazole (fungicides) and Triazophos (insecticide) are used to control leaf spot and leaf minors respectively in guar [8]. The plant absorbs a certain amount of the pesticides applied; changes in the plant's metabolism take place. In connection with this the impact of Hexaconazole and Triazophos on several metabolism of legume vegetable, guar has not yet been studied. Hence the attempt had made to study the effect of these pesticides on ureide contents in guar after seed treatment and foliar sprays.

Materials and Methods

The seed soaking treatments of Hexaconazole 5% EC and Triazophos 40% EC were given separately to healthy seeds of guar. The seeds were soaked in 0.05, 0.1, 0.15, 0.2 and 0.3% (v/v) concentrations of these pesticides for 12 h. After 12 h seed soaking period, the treated seeds were thoroughly washed with distilled water and sown in earthen pot containing garden soil and manure (3:1). The seeds treated with distilled water for 12 hour and sown in earthen pot were used as control.

*Corresponding author: Yuvraj D Kengar, Department of Botany, Kanya Mahavidyalaya, Karad, Maharashtra India, Tel: 02342 224094; E-mail: yuvrajkengar@gmail.com

Received December 13, 2016; Accepted February 05, 2017; Published March 01, 2017

Citation: Kengar YD, Patil BJ (2017) Ureide Content of Guar under Influence of Hexaconazole and Triazophos. J Fertil Pestic 8: 176. doi:10.4172/2471-2728.1000176

Copyright: © 2017 Kengar YD, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

The first foliar spray of Hexaconazole and Triazophos were applied with respective concentrations on 10th day while second foliar spray was on 25th day of plant growth. Analysis of ureide was carried out on the 15th and 30th day of growth i.e., 5 days after each spray.

Ureide content was measured according to the method of [11]. One hundred mg of dried and powdered plant material was taken in a test tube followed by 2 ml of 0.25 N NaOH. It was stirred and heated for 10 min at 100°C. After adding 1 ml of 0.65 N HCl at 100°C again it was heated for 10 min at 100°C. 1 ml of 0.4 M sodium phosphate buffer (ph 7.0) was added and it was cooled to room temperature and centrifuged for 5 min at 400 rpm. 1.5 ml supernatant was mixed with 0.25 ml of fresh phenylhydrazine solution (0.1 g of phenylhydrazine HCl, dissolved in 30 ml distilled water, prepared few minute before use. It was kept at room temperature for 5 minutes and then cooled on an ice bath. It was mixed with 1.25 ml pre-cooled concentrated HCl followed by 0.25 ml ferricyanide solution (0.50 g potassium ferricyanide in 30 ml distilled water)). Then the sample was removed from the ice bath and absorbance of colored product (dibenzyl formazan) was read at 535 nm after 15 min. The ureide content was estimated with the help of standard curve of Allantoin (0.05 mg ml⁻¹) using different concentrations.

Results

Effect of Hexaconazole after seed treatment and foliar sprays on ureide content in guar is studied and results are recorded in Table 1. The ureide content was increased after first foliar spray of Hexaconazole however it was decreased after second foliar sprays of different concentrations. The remarkable increased values of ureide were recorded at 0.10, 0.15 and 0.20% Hexaconazole (12.39, 14.12 and 16.48 μ mols g⁻¹) in guar after first foliar spray. The higher doses of Hexaconazole were act much detrimental on ureide content after second spray. The 0.30% Hexaconazole second spray recorded 2.93 μ mols g⁻¹ ureide content in guar which is much reduced as compared to control; whereas 0.05 to 0.20% Hexaconazole reported 18.75, 16.43, 14.54 and 13.38 μ mols g⁻¹ ureide content which was also less than control.

The effect of Triazophos on ureide content in guar was studied and it is recorded in Table 1. The ureide content in guar was decreased after both foliar sprays of Triazophos. The ureide content recorded at 0.05 to 0.30% Triazophos were 10.32, 10.39, 9.12, 6.48 and 4.37 μ mols g⁻¹ after first foliar spray whereas 14.78, 13.18, 11.56, 8.10 and 6.75 μ mols g⁻¹ after second foliar sprays in guar. The ureide content was reduced after treatment of Triazophos in all doses; however 0.20 and 0.30%

Treatment	Ureide content (μ mols g ⁻¹ dry weight)	
	First foliar spray	Second foliar spray
Control	11.23	19.23
Hexaconazole (% V/V)		
0.05	10.66	18.75
0.10	12.39	16.43
0.15	14.12	14.54
0.20	16.48	13.38
0.30	11.37	2.93
Triazophos (% V/V)		
0.05	10.32	14.78
0.10	10.39	13.18
0.15	9.12	11.56
0.20	6.48	8.10
0.30	4.37	6.75

Table 1: Effect of hexaconazole and triazophos seed treatment and foliar spray on ureides content of Guar.

Triazophos recorded much reduced ureide content after seed treatment and foliar sprays.

In general, the ureide content increased only after first foliar spray of Hexaconazole, whereas in case of Triazophos treatment, the ureide content showed a decreased value with increased concentrations after both the spray.

Discussion

Leguminous plants are categorized into two groups depending upon the transporting form of nitrogen, one includes ureide transporting plants and other consists of amide transporting plants. Ureide contents in the oldest, primary leaves, and in the youngest, leaves during the development of plants grown under nitrogen fixation conditions or of nitrate-fertilized have been studied by many workers [12]. The activity of Allantoinase reported in the primary and the uppermost leaves during the development of nitrogen-fixing plants and plants grown with nitrate as the main nitrogen source. The ureides, allantoin and allantoate are major forms of nitrogen transported from root nodules to shoots in tropical legumes. In these plants, nitrogen fixed is used for purine synthesis. Through a series of enzymatic steps, purines are oxidized to allantoin and allantoate. Ureides synthesized in the nodules are transported to the shoot where they should be degraded and their N content assimilated. De novo purine synthesis is the main route for ureide formation in nodules. However, purines involved in the biogenesis of ureides may also arise by turnover of nucleic acids [13]. However, the biosynthetic route, degradation of ureides starts with hydrolysis of the internal amide bond of allantoin which giving rise to allantoate, reaction catalysed by allantoin amidohydrolase, characterized in plants [14,15]. The pathway for degradation of allantoate into glyoxylate and ammonia, is still under debate and further work. The occurrence of several pathways for the degradation of both allantoate and ureidoglycolate has been reported [16,17]. Most recent reports suggested that plants degrade allantoate to ureidoglycolate via allantoate amidohydrolase (AAH; EC 3.5.3.9) and ureidoglycine aminohydrolase (EC 3.5.3.-) [18-22]. The ureidic plants relying upon N₂ fixation as the sole nitrogen source, ureides may comprise up to 86% of the N in the xylem sap, whereas amino acids, amides and nitrate are the major forms of nitrogen translocated from the roots after fertilization with nitrate [23,24]. In these plants, it is assumed that ureides reach high concentrations only in nodulated, nitrogen-fixing plants stem or petiole. This ureide levels has been established as an easy method to determine nitrogen fixation rates [24-28]. There are several reports h showing plant development influences the level of ureides in xylem sap and in leaves [29,30]. Changes in ureide levels upon plant development have been considered an important factor for the use of the ureide as a convenient method to determination of rate of nitrogen fixation [31].

In our experiments, the ureide content was increased after first foliar spray of Hexaconazole however it was decreased after second foliar sprays of different concentrations. The remarkable increased values of ureide were recorded at 0.10 to 0.20% Hexaconazole after first foliar spray. The higher doses of Hexaconazole were act much detrimental on ureide content after second spray. The second spray of 0.30% Hexaconazole responsible for much reduction of ureide content as compared to control. The ureide content in guar was decreased after both foliar sprays of Triazophos. However the 0.20 and 0.30% Triazophos recorded much reduced ureide content after seed treatment and foliar sprays. In general, the ureide content increased only after first foliar spray of Hexaconazole, whereas in case of Triazophos treatment, the ureide content showed a decreased value with increased

concentrations after both the spray. The higher doses reduced the ureide content in guar. This indicated that the pesticides impose stress on ureide metabolism at higher doses. However the ureide levels have been shown to rise under water stress conditions also, and it has been suggested that the accumulation of ureides responsible for the feedback inhibition of nitrogen fixation in adverse conditions [32,33]. Recently reported that ureide content increases considerably in nonnodulated common bean plants suffering water stress and drought-induced senescence was considered the possible source of ureides [5]. An increase in ureide content of soybean leaves has been reported by Yukimoto and Ishianj after application of some organophosphorus insecticides [34].

The first spray of Hexaconazole was act as inducer for enzymatic system while excess doses may act suppressor in guar. The Triazophos retards the regulation mechanism of ureide synthesis indicated the inactivation of enzymes involved in this metabolism [35]. However there is little information on the regulation of genes and enzyme activities of ureide metabolism. Moreover regulation of ureide metabolism gene expression has been investigated in a ureidic plants [36,37] will help to focus on more details about ureide metabolism in guar like plants to glance on the nitrogen fixation mechanism.

References

1. Streeter JG (1991) Transport and metabolism of carbon and nitrogen in legume nodules. *Advances in Botanical Research* 18: 129-187.
2. Walsh KB (1995) Physiology of the legume nodule and its response to stress. *Soil Biology and Biochemistry* 27: 637-655.
3. Schubert S, Serraj R, Plies BE, Mengel K (1995) Effect of drought stress on growth, sugar concentrations and amino acid accumulation in N₂-fixing alfalfa. *Journal of Plant Physiology* 146: 541-546.
4. Herridge DF, Peoples MB (1990) Ureide assay for measuring nitrogen fixation by nodulated soybean calibrated by N methods. *Plant Physiology* 93: 495-503.
5. Alamillo JM, Diaz LJJ, Sanchez MMV, Pineda M (2010) Molecular analysis of ureide accumulation under drought stress in *Phaseolus vulgaris* L. *Plant, Cell and Environment* 33: 1828-1837.
6. Fischinger SA, Drevon JJ, Claassen N, Schulze J (2006) Nitrogen from senescing lower leaves of common bean is re-translocated to nodules and might be involved in a feedback regulation of nitrogen fixation. *Journal of Plant Physiology* 163: 987-995.
7. Serraj R, Vadez VV, Denison RF, Sinclair TR (1999) Involvement of ureides in nitrogen fixation inhibition in soybean. *Plant Physiology* 119: 289-296.
8. Kengar YD, Kamble AB, Sabale AB (2014) Effect of hexaconazole and triazophos on seed germination and growth parameters of spinach and guar. *Annals of Biological Research* 5: 89-92.
9. Ahemad M, Khan MS (2010) Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting *Rhizobium leguminosarum*. *Crop Protection* 29: 325-329.
10. Jerlin B (2001) Effects of atrazine on growth nodulation and nitrogen constituents of *Vigna mungo*. *J Ecotoxicol Environ Monit* 11: 209-214.
11. Glenister RA, Rue JL (1987) Measuring ureides in symbiotic nitrogen fixation. INC, New York.
12. Juan LDL, Gregorio GV, Javier F, Manuel P, Josefa MA (2012) Developmental effects on ureide levels are mediated by tissue-specific regulation of allantoinase in *Phaseolus vulgaris* L. *Journal of Experimental Botany* 63: 4095-4106.
13. Zrenner R, Stitt M, Sonnewald U, Boldt R (2006) Pyrimidine and purine biosynthesis and degradation in plants. *Annual Review of Plant Biology* 57: 805-836.
14. Webb MA, Lindell JS (1993) Purification of allantoinase from soybean seeds and production and characterization of anti-allantoinase antibodies. *Plant Physiology* 103: 1235-1241.
15. Yang J, Han KH (2004) Functional characterization of allantoinase genes from *Arabidopsis* and a nonureide-type legume black locust. *Plant Physiology* 134: 1039-1049.
16. Todd CD, Tipton PA, Blevins DG, Piedras P, Pineda M, et al. (2006) Update on ureide degradation in legumes. *Journal of Experimental Botany* 57: 5-12.
17. Munoz A, Bannenberg GL, Montero O, Cabello DJM, Piedras P, et al. (2011) An alternative pathway for ureide usage in legumes: enzymatic formation of a ureidoglycolate adduct in *Cicer arietinum* and *Phaseolus vulgaris*. *Journal of Experimental Botany* 62: 307-318.
18. Todd CD, Polacco JC (2006) ATAAH encodes a protein with allantoinamidohydrolase activity from *Arabidopsis thaliana*. *Planta* 223: 11080-1113.
19. Werner AK, Sparkes IA, Romeis T, Witte CP (2008) Identification, biochemical characterization, and subcellular localization of allantoinamidohydrolases from *Arabidopsis* and soybean. *Plant Physiology* 146: 418-430.
20. Werner AK, Romeis T, Witte CP (2010) Ureide catabolism in *Arabidopsis thaliana* and *Escherichia coli*. *Nature Chemical Biology* 6: 19-21.
21. Werner AK, Witte CP (2011) The biochemistry of nitrogen mobilization: purine ring catabolism. *Trends in Plant Science* 16: 381-317.
22. Serventi F, Ramazzina I, Lamberto I, Puggioni V, Gatti R, et al. (2010) Chemical basis of nitrogen recovery through the ureide pathway: formation and hydrolysis of S-ureidoglycine in plants and bacteria. *ACS Chemical Biology* 19: 203-214.
23. McClure PR, Israel DW (1979). Transport of nitrogen in the xylem of soybean plants. *Plant Physiology* 64: 411-416.
24. McClure PR, Israel DW, Volk RJ (1980) Evaluation of the relative ureide content of xylem sap as an indicator of N₂ fixation in soybeans: greenhouse studies. *Plant Physiology* 66: 720-725.
25. Pate JS, Atkins CA, White ST, Rainbird RM, Woo KC (1980) Nitrogen nutrition and xylem transport of nitrogen in ureide-producing grain legumes. *Plant Physiology* 65: 961-965.
26. Herridge DF (1982) Use of the ureide technique to describe the nitrogen economy of field-grown soybeans. *Plant Physiology* 70: 7-11.
27. Patterson TG, LaRue TA (1983) Nitrogen fixation (C₂H₂) and ureide content of soybeans: Ureides as an index for fixation. *Crop Science* 23: 825-831.
28. Herridge DF, Bergersen FJ, Peoples MB (1990) Measurement of nitrogen fixation by soybean in the field using the ureide and natural ¹⁵N abundance methods. *Plant Physiology* 93: 708-716.
29. Aveline A, Crozat Y, Pinochet X, Domenach AM, Cleyet MJC (1995) Early remobilization: a possible source of error in the ureide assay method for N₂ fixation measurement by early maturing soybean. *Soil Science and Plant Nutrition* 41: 737-751.
30. Schubert KR (1986) Products of biological nitrogen fixation in higher plants: Synthesis, transport and metabolism. *Annu Rev Plant Physiol* 37: 539-574.
31. Vankessel C, Roskoski JP, Keane K (1988) Ureide production by N₂-Fixing and non N₂-Fixing leguminous trees. *Soil Biol Biochem* 20: 891-897.
32. Serraj R (2003) Effects of drought stress on legume symbiotic nitrogen fixation: physiological mechanisms. *Indian Journal of Experimental Biology* 41: 1136-1141.
33. King CA, Purcell LC (2005) Inhibition of N₂ fixation in soybean is associated with elevated ureides and amino acids. *Plant Physiology* 137: 1389-1396.
34. Yukimoto M, Ishianj A (1981) Phytotoxicities of organophosphorus insecticides to crops.6. Nitrogen contents in soybean leaves applied with organophosphorus insecticides. *Chem Insp Stn Tokyo* 21: 50-53.
35. Tu CM (1981) Effects of pesticides on activities of enzymes and microorganisms in a clay soil. *J Environ Sci Health B* 16: 179-191.
36. Charlson DV, Korth KL, Purcell LC (2009) Allantoinamidohydrolase transcript expression is independent of drought tolerance in soybean. *Journal of Experimental Botany* 60: 847-851.
37. Yang SS, Valdes LO, Xu WW, Bucciarelli B, Gronwald JW, et al. (2010) Transcript profiling of common bean (*Phaseolus vulgaris* L.) using the GeneChip Soybean Genome Array: optimizing analysis by masking biased probes. *BMC Plant Biology* 10: 85.