



Antityrosinase activity and photosynthetic pigments in seaweed treated sprouts of *Vigna aconitifolia*

NIDHI VERMA¹, KRISHAN D SEHRAWAT², SANGEETA KUMARI³ and ANITA R⁴

Maharshi Dayanand University, Rohtak, Haryana 124 001, India

Received: 11 June 2019; Accepted: 22 July 2019

ABSTRACT

The seaweed, *Ascophyllum nodosum* is the focus of the research these days and is looked upon as an important alternative bio-fertilizer. For this purpose the interaction of the seaweed with different plants including legumes is important to understand. We previously noticed lesser pigmented nodules in legume plant *Vigna aconitifolia*, when grown in presence of *A. nodosum* Extract (ANE). We therefore studied the effect of the ANE on the tyrosinase inhibition activity, an enzyme known to produce melanin pigment. In addition, the effect of the treatment of ANE on the photosynthetic pigments was also recorded. The seeds of *Vigna aconitifolia* were treated with 0%, 0.01%, 0.05%, 0.10%, 0.50% and 1.0% of *A. nodosum* extract for different time periods from 0–24 hours. It was observed that with the increased exposure to the seaweed (ANE), the tyrosinase inhibition activity also increased up to 0.05% of the seaweed concentration at 24 h of soaking and decreased after increasing the concentration of ANE from 0.1–1.0% at the same time period. Seed treatment with 0.05% seaweed was found to be the most effective concentration showing highest tyrosinase inhibition.

Key words: *Ascophyllum nodosum*, Photosynthetic pigments, Tyrosinase inhibition, *Vigna aconitifolia*

Seaweed extract is one of the successful biofertilizer that has been commercialized to use in agriculture as these have high nutritive value as compared to the chemical fertilizers. Among many species of seaweeds, *Ascophyllum nodosum* is thoroughly studied for its use in agriculture (Craigie *et al.* 2010). The *A. nodosum* contains organic acids, macronutrients, micronutrients and various hormones, viz. cytokinins, auxins, gibberellins, betaines, mannitol and proteins that are important for the crop and plant growth (Norrie *et al.* 1999). Edible seaweeds have been known to certain antioxidant (Kuda *et al.* 2005, Kumar *et al.* 2008) and tyrosinase inhibitory activity (Yoon *et al.* 2009).

Vigna aconitifolia (moth bean/dew bean/matki) is a minor pulse crop. Like other pulses, it supplies fair share of proteins to a vegetarian diet. Tyrosinase (E.C. 1.14.18.1) is a copper-containing oxidase enzyme present in plant as well as animal tissues that produce melanin and other pigments from tyrosine by oxidation (Anisimov *et al.* 2014). It catalyzes oxidation of phenolic substrates to o-quinones, which are then polymerized to brown, red or black pigments

(Friedman *et al.* 1996). These quinines irreversibly react with amino and sulfhydryl groups of protein which decreases the digestibility of protein and bioavailability of amino acids including lysine, cysteine (Ando *et al.* 2007). The increase in tyrosinase activity resulted in the increase in the melanin pigment known as melanoma. Hence, the decrease in tyrosinase activity (tyrosinase inhibition) is important for the prevention of skin related diseases like, melasma, hyper pigmentation, and liver spot (Ando *et al.* 2007). Several synthetic tyrosinase inhibitors have been developed but exhibited lack of efficiency or adverse side effects (Hamon *et al.* 2009). For this reason, it is necessary to search and develop novel tyrosinase inhibitors with potent activity and fewer side effects. Our previous work suggested depigmentation in the nodules of *V. aconitifolia* when grown in presence of the seaweed, *A. nodosum*. The present study is an attempt to understand the tyrosinase inhibition abilities of the seaweed, if any. In addition, the carotenoid and other photosynthetic pigments were also estimated at early to look for the effect of the seaweed on overall pigmentation per se.

MATERIALS AND METHODS

The experiment was conducted at the Department of Botany, M D University, Rohtak during 2017–18. Seaweed, *Ascophyllum nodosum* (trade name: Biovita, PI industries, Udaipur, Rajasthan) and the seeds of *Vigna*

Present address: ¹, ³Ph D Scholars (nidhi.verma40@gmail.com, sangeetahalawat@gmail.com), ⁴Professor (anitarshrawat@gmail.com), Department of Botany, Maharshi Dayanand University, Rohtak, Haryana, India; ²Assistant Scientist (krishanssehrswat@gmail.com), Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar.

aconitifolia (RMO 225) were purchased from Rajasthan, India. Chemicals; Mercuric chloride, Acetone, L-Dopa and Potassium phosphate monobasic and dibasic were purchased from Himedia. The enzyme Tyrosinase and its inhibitor Kojic acid were purchased from Sigma Aldrich.

Seaweed treatment: The seaweed, *Ascophyllum nodosum* extract (ANE) was diluted to 0.01%, 0.05%, 0.10%, 0.50% and 1.0% (v/v) with sterilized water. Only sterile distilled water was taken as negative control. Ten gram of seeds was sterilized with 0.1% of mercuric chloride followed by 2–3 washing with distilled water. The seeds were soaked in 20 ml of different concentrations of seaweed for 12 h (i.e. 0 hour of sprouting) in a 100 ml beaker. After soaking the seaweed was discarded and seeds were left in petri plates containing the different concentrations of seaweed for sprouting for 0, 6, 12, 18 or 24 h.

Tyrosinase inhibition activity assay: One gram of seeds sprouted for different time periods of all above treatment with and without seaweed was crushed in 2.0 ml of 80% methanol and were stirred on an orbital shaker at 200 rpm for 10 min at room temperature. The crushed material was placed in water bath for 10 min having 60°C temperature and centrifuged it at 10000 rpm for 10 min. The supernatant was taken in a tube and the pellet was again washed with 2 ml of 80% methanol. The supernatant was collected for tyrosinase inhibition assay (Yao *et al.* 2011- with slight modifications). In a sterile clean tube, 40 µl of 50 mg/ml plant extract, 80 µl of 0.1 M (pH-6.8) sodium phosphate buffer, 40 µl of tyrosinase enzyme (31 units) and 40 µl of L-dopa (2.5 mM) were added step by step. The reaction mixture was incubated for 30 min and then the absorbance was measured at 475 nm. The control group had all the constituents except for the plant extract. Kojic acid (0.025 gm/mL) was used as a positive control of tyrosinase inhibition.

Determination of chlorophyll content: One gram of seeds harvested at different time periods following the above-mentioned treatments with and without seaweed. The seeds were crushed in 5 ml of 80% acetone and centrifuged at 5000 rpm for 15 min. The supernatant was stored at 4°C until used. The pellet was again extracted with 5 ml of 80% acetone and the extracted pigments were pooled and used for photosynthetic pigment determination (Arnon 1949). Absorbance was read at 645 and 643 nm in Thermo Scientific Evolution 201 UV-visible spectrophotometer. The chlorophyll a, Chlorophyll b and Total Chlorophyll were estimated.

Determination of carotenoid content: It was determined by using protocol of Kirk and Allen (1965). The acetone extract obtained as extract in the methods described in the previous section was also used for the measurement of carotenoid content. The absorbance was read at 480 nm in Thermo Scientific Evolution 201 UV-Visible Spectrophotometer.

Statistical analysis: The difference obtained in the Tyrosinase inhibition assay and that of the Photosynthetic pigments was tested for significance using one way analysis of variance (ANOVA). Further the differences between the

groups were tested by employing the HSD Tukey test. The P-value less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Tyrosinase inhibition activity: Treatment of *V. aconitifolia* seeds with the sea weed, *A. nodosum* prior to germination led to the depigmentation of the nodules (communicated elsewhere). The findings may be suggestive of the depigmentation potential of the seaweed in the plants. The *V. aconitifolia* seeds were soaked in the seaweed for 12 h and the tyrosinase inhibition was recorded in the subsequent 24 h at a regular time interval of 6 h each. The inhibition of the tyrosinase activity was found to be maximum at 24 h of sprouting Fig 1 (ANOVA; F= 840.3; P <0.0001). The activity was increased from 0–24 h (HSD Tukey; P <0.01).

Among different concentration of seaweed the maximum tyrosinase inhibition activity was found at 0.05% concentration of seaweed (Fig 1) (ANOVA; F= 735.3; P <0.0001). The depigmentation observed in the nodules of *A. nodosum* was maximum when treated with 0.05% of the seaweed at 24 h. Marine derived active ingredients are used in cosmetic industry because of their beneficial impact on human skin. Among all seaweeds the brown seaweed has great value in cosmetic industry because they grow in areas where light is more.

The tyrosinase inhibition activity was very less studied in legumes. However, the legume itself is known to have tyrosinase inhibition potential. The tyrosinase inhibition was first reported in *Vigna mungo* (Zia-Ul-Haq 2014). The increase in the tyrosinase inhibition was also reported in *Vigna radiata* upon sprouting (Jeong 2016). Yao *et al.* (2011) studied the tyrosinase inhibition activity in 16 legumes and found maximum tyrosinase inhibition activity in mung bean with inhibition percentage of 81.24%. Kim *et al.* (2012) reported tyrosinase inhibition activity in mung bean sprouts using different solvents and found ethanol extract showed maximum activity followed by n-BuOH and n-hexane extracts. In East Asian countries the extract from roots and seeds of *Glycyrrhiza* species (Leguminosae) are used as effective ingredient for skin-whitening agents (Yokota *et al.* 1998). In cosmetic production moth bean extract has high stability; it maintains its quality longer even when come in contact with atmosphere (Contet-Audonneauet *et al.* 2005). Potential antityrosinase activity was reported in moth bean by Novoa *et al.* (2015). The strong tyrosinase inhibitory activity of *Grateloupia lancifolia* (Red algae) extract could decrease the amount of melanin (Yoon *et al.* 2009, Kim *et al.* 2012).

Pigment determination: The maximum chlorophyll 'a' (ANOVA; F= 1575; P <0.0001), chlorophyll 'b' (ANOVA; F=9005.75; P <0.0001) and total carotenoid content (ANOVA; F= 820.79; P <0.0001) was found at 0 h of sprouting.

With subsequent increase in the time interval, the chlorophyll 'a', chlorophyll 'b' and total carotenoid content was decreased in the moth bean (Fig 2). The reason of decreasing carotenoid content may be attributed to the fact

that after soaking, the soluble pigments and anthocyanin leached out from the seed coats. The dry seeds are known to have high carotenoid content than soaked seed or sprouts.

Further, carotenoid content decreases upon germination for 1 day following which the carotenoid content gradually increases (Ghazali and Cheng 1991). Seaweeds are known source of natural pigments (Chinnadurai *et al.* 2013). In present study, we found that with the increase in the seaweed concentration all the photosynthetic pigments, i.e. chlorophyll 'a', chlorophyll 'b' and total carotenoid content, increased till 0.05% of seaweed. Further increase in the concentration beyond 0.05% seaweed, however, led to a decrease in the content of chlorophyll 'a' (ANOVA; $F = 24984.04$; $P < 0.0001$), chlorophyll 'b' (ANOVA; $F = 169068.5$; $P < 0.0001$) and total carotenoid (ANOVA; $F = 8907.01$; $P < 0.0001$). The concentrations of photosynthetic pigments are reported to increase in the presence of the moderate concentration of seaweeds (Whapham *et al.* 1993, Thirumaran *et al.* 2009, Kumari *et al.* 2011). Higher concentration of seaweed causes browning of cotyledons results from loss of chloroplast integrity (Wu and Lin 2000). The results of our study showed that with increased time period's tyrosinase inhibition activity was increased but the photosynthetic pigments decreased. Among different concentrations of *Ascophyllum nodosum*, the 0.05% concentration was the best for both tyrosinase inhibition activity as well as the abundance of the photosynthetic pigments (Fig 3). Further study is required to explore phytochemicals from moth bean and their bioactive capacities to understand the underlying mechanism of depigmentation.

REFERENCES

- Ando H, Kondoh H, Ichihashi M and Hearing V J. 2007. Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase. *Journal of Investigative Dermatology* **127**(4): 751–61.
- Anisimov M M, and Chaikina E L. 2014. Effect of seaweed extracts on the growth of seedling roots of soybean (*Glycine max* (L.) Merr.) seasonal changes in the activity. *International Journal of Current Research and Academic Review* **2**(3): 19–23.
- Arnon D I. 1949. Copper enzymes in isolated chloroplasts. Photo phenol oxidase in *Beta vulgaris*. *Plant Physiology* **24**: 1–15.
- Chinnadurai S, Kalyanasundaram G, Chermampandi P, Annadurai H, and Perumal A. 2013. Estimation of major pigment content in seaweeds collected from Pondicherry coast. *Experiment* **9**(1): 522–5.
- Contet –Audonneau J L, Louis D, Gillon V, Pauly G, and Moser P. 2005. Use of an extract from the *Vigna aconitifolia* plant in a cosmetic and ordermo pharmaceutical composition. Cognis corporation patent department 300 brookside avenue ambler, pa 19002 (us).
- Craigie J S. 2010. Seaweed extracts stimuli in plant science and agriculture. *Journal of Applied Phycology* **23**: 371–93.
- Friedman M. 1996. Food browning and its prevention: An overview. *Journal of Agriculture Food Chemistry* **44**: 631–53.
- Ghazali H M and Cheng S C. 1991. The effect of germination of the physico-chemical properties of black gram (*Vigna mungo* L.). *Food Chemistry* **41**(1): 99–106.
- Hamon V L M and Criton M. 2009. Phenylethylamide and phenylmethylamide derivatives as new tyrosinase inhibitors. *Biological and Pharmaceutical Bulletin* **32**: 301–3.
- Jeong Y M, Ji H H, Noh G Y and Park S N. 2016. Inhibitory effects of mung bean (*Vigna radiata* L.) seed and sprout extracts on melanogenesis. *Food Science and Biotechnology* **25**(2): 567–73.
- Kim D K, Jeong S C, Gorinstein S and Chon S U 2012. Total polyphenols, antioxidant and antiproliferative activities of different extracts in mungbean seeds and sprouts. *Plant Foods for Human Nutrition* **67**(1): 71–5.
- Kirk J T O and Allen R L. 1965. Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione. *Biochem. Biophysics Research Journal of Canada* **27**: 523–30.
- Kuda T, Tsunekawa M, Goto H and Araki Y. 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula. *Japan Journal of Food Composition and Analysis* **18**: 625–33.
- Kumar K S, Ganesan K and Rao P V S. 2008. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii*. *Food Chemistry* **107**: 289–95.
- Kumari R, Kaur I and Bhatnagar A K. 2011. Effect of aqueous extract of *Sargassum johnstonii* Setchell & Gardner on growth, yield and quality of *Lycopersicon esculentum* Mill. *Journal of Applied Phycology* **23**(3): 623–33.
- Norrie J and Hiltz D A. 1999. Seaweed extracts research and applications in agriculture. *Agro-Food-Industry Hi-Tech*: 15–8.
- Novoa D E G, Favaro R, Silvmo T S T, Ribeiro F C N, Santos R M and Costa A. 2015. Menopause and cosmeceuticals-Skin, Mucosa and Menopause. Springer-Verlag, Berlin, Heidelberg, Germany 455–78.
- Thirumaran G, Arumugam M, Arumugam R and Anantharaman P. 2009. Effect of seaweed liquid fertilizer on growth and pigment concentration of *Abelmoschus esculentus* (l) medikus. *American-Eurasian Journal of Agronomy* **2**(2): 57–66.
- Whapham C A, Blunden G, Jenkins T and Hankins S D 1993. Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *Journal of Applied Phycology* **5**: 231–34.
- Wu Y T and Lin C H. 2000. Analysis of cytokinin activity in commercial aqueous seaweed extract. *Gartenbuwis senschaft* **65**: 170–73.
- Yao Y, Cheng X, Wang L, Wang S and Ren G 2011. Biological potential of sixteen legumes in China. *International Journal of Molecular Sciences* **12**(10): 7048–58.
- Yokota T, Nishio H, Kubota Y and Mizoguchi M. 1998. The inhibitory effect of glabridin from licorice extracts on melanogenesis and inflammation. *Pigment Cell Research* **11**: 355–61.
- Yoon N Y, Eom T K, Kim M M and Kim S K 2009. Inhibitory effect of phlorotannins isolated from *Ecklonia cava* on mushroom tyrosinase activity and melanin formation in mouse B16F10 melanoma cells. *Journal of Agriculture Food Chemistry* **57**: 4124–9.
- Zia-Ul-Haq M, Ahmad S, Bukhari S A, Amarowicz R, Ercisli S and Jaafar H Z E 2014. Compositional studies and biological activities of some mash bean (*Vigna mungo* (L.) Hepper) cultivars commonly consumed in Pakistan. *Biological Research* **47**(1): 1–14.