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Influence of cyanobacterial auxin on sprouting of taro (*Colocasia esculenta* var. *antiquorum*) and corm yield

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ABSTRACT

Cyanobacteria are widely exploited in wet land rice cultivation as biofertilizer. Apart from nitrogen fixation, cyanobacteria are capable of producing a number of plant growth promoting substances. Among this, Indole acetic acid (IAA) is of prime importance. In the present study, five strains of nitrogen fixing filamentous cyanobacteria, viz. *Anabaena, Aulosira, Cylindrospermum, Nostoc* and *Tolypothrix* were tested for IAA production. IAA production in cyanobactreia could be induced by culturing them in tryptophan supplemented BG11 media. IAA was detected and quantified using Salkowski's method. Among the five strains *Aulosira, Nostoc* and *Tolypothrix* produced significant amount of IAA. Response surface methodology (RSM) was used to optimise three parameters, viz. tryptophan, pH and culture period to improve IAA production by *Nostoc*. The results revealed that the selected parameters had a significant effect on IAA production. Based on the RSM results under optimum conditions, viz. tryptophan 2.75 mg/ml, pH 7 and incubation time 14 days, *Nostoc* produced 8.66 µg/ml IAA. The culture filtrate of *Nostoc* effectively promoted sprouting of taro corm and the induction effect was ascertained on germination of cowpea seeds. The biometric parameters of taro corm and cowpea seeds exposed to *Nostoc* culture filtrate showed the growth promoting attribute of the cyanobacteria compared to that of synthetic IAA. This study reports for the first time the possible application of cyanobacteria as a biofertilizer to two varieties, viz. Muktakeshi and Telia of a tuber crop taro (*Colocasia esculenta*) adapted to waterlogged conditions.

Key words: Auxin, Blue green algae, Box-Behnken statistical design, Response Surface Methodology, Salkowski's test, Tryptophan

Products of microbial metabolism like amino acids, auxins, gibberellic acid etc., are reported to be positively influencing plant growth and productivity. Plant growth promoting rhizobacteria (PGPRs) have been reported to maintain soil quality and increase yield by nitrogen assimilation, IAA production and growth promotion in rice (Amprayn *et al*. 2012, Anizan *et al*. 2012), common bean (Mazen *et al*. 2008, Khalequzzaman and Hossain 2008, Tajini *et al*. 2012, Hungria *et al*. 2015, Pankaj Kumar *et al*. 2016) and greengram (Sindhu *et al*. 2002). Akin to rhizobacteria, cyanobacteria are photosynthetic prokaryotes (blue-green algae) ubiquitously distributed in aquatic ecosystems and play pivotal role in nutrient cycling. Cyanobacteria range in their morphology from unicellular to differentiated and branched types. Cyanobacteria produce good amount of biomass, have high growth rate, require only traces of elements for their survival, produce an array of plant growth stimulating and plant protecting molecules (Manjunath *et al*. 2010, Hashtroudi *et al*. 2013). Cyanobacteria are capable of producing wide variety of compounds like amino acids, sugars, vitamins and plant growth regulators which directly or indirectly influence plant growth (Prasanna *et al*. 2008, Shariatmadari *et al*. 2013). Some members of this group are diazotrophs, capable of fixing atmospheric nitrogen (Vincent 2009). The application of nitrogen fixing cyanobacteia will increase soil nitrogen content and hence recommended as biofertilizers (Mishra and Sunil Pabbi 2004, Saadatnia and Riahi 2009, Paudel *et al*. 2012). Apart from this, cyanobacteria affect physico-chemical properties of soil such as increasing top soil aggregates (Issa *et. al*. 2007), soil water retention (Eldridge and Greene 1994), soil organic carbon, and available nutrients (Maqubela *et al*. 2008). Beneficial effects of cyanobacteria have been reported for rice, wheat, soybean, oat, tomato, radish, cotton, sugarcane, maize, chilli, bean, muskmelon, lettuce and cucumber (Cano de Storni *et al*. 2002, Mishra and Sunil Pabbi 2004, Maqubela *et al*. 2008, Saadatnia and Riahi 2009, Hussain and Hasnain 2011, Paudel *et al*. 2012, Shariatmadari *et al*. 2013, Menamoa and Wolde 2013, Priya *et al*. 2015). Nevertheless, the aforementioned beneficial effects of cyanobacteria have not been studied in taro (*Colocasia esculenta*) an important tropical tuber crop grown world-wide under lowland conditions.

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Indole Acetic Acid (IAA) is a growth regulator produced by young leaves, stem and seeds of plants. IAA play significant role in the growth and development of plants. IAA regulates epical dominance, development of adventitious roots, phototropism, geotropism, and also prevent abscission of leaves and roots. IAA production is extensively present in soil microorganisms especially plant associated microorganisms.

Taro is a perennial herb with clusters of long heart- or arrowhead-shaped leaves that point downwards. It is a widely cultivated and consumed tuber crop all over India particularly in North-Eastern states (Sethuraman Sivakumar *et al*. 2013a,b). Taro corm- used as a staple food, as a side dish, or as an ingredient in various side dishes- is an excellent source of starch and dietary fibre (Sethuraman Sivakumar *et al*. 2013a,b). Young leaves of taro are rich in vitamin C and the corms are rich in starch and vitamin B complexes. Taro is cultivated in hydromorphic soils or flooded conditions; it is one of the few crops that can be grown under flooded conditions. Scarcity and rising cost of chemical fertilizers are major constraints of crop production. This necessitates the development of an alternative methodology to improve crop production. In this scenario, organic farming and biofertilizers are gaining importance as suitable alternative for chemical fertilisers and for sustainable agricultural production. Organic sources of nutrients could sustain taro yield at par with conventional practice of cultivation (Suja *et al*. 2015). Application of biofertilizers such as *Azospirillum*, mycorrhiza and phosphobacteria along with organic source of nutrients could sustain taro yield at par with conventional practice of cultivation in an economic and eco-friendly manner (Suja *et al*. 2015). We report for the first time the efficiency of nitrogen fixing filamentous cyanobacteria in growth regulator production (IAA) and corm sprouting stimulation in two varieties, viz. Muktakeshi and Telia of taro (*Colocasia esculenta*).

MATERIALS AND METHODS

Five nitrogen fixing filamentous cyanobacteria - *Anabaena* (CCC 660), *Aulosira* (CCC662), *Cylindrospermum* (CCC 665), *Nostoc* (CCC670) and *Tolypothrix* (CCC679) procured from the Centre for Conservation and Utilisation of Blue Green Algae (CCUBGA), Indian Agricultural Research Institute, New Delhi, India were used for the study conducted during 2013-2015. The cyanobacteria were cultured in BG 11 medium under 1500 lux light intensity, 25 ± 2°C with 8/16 hr light /dark cycle. One taro land race Telia and one variety Muktakesi which are well adapted to water-logged conditions were used in this study.

For IAA production, cyanobacteria were inoculated in to nitrogen free BG11 medium supplemented with 1 mg/ ml tryptophan in aseptic conditions and incubated in 8h:16h (light: dark) cycle for 15 days. To study IAA production in the absence of the precursor, tryptophan-free media were also used. The supernatant of the cyanobacteria culture medium was collected by centrifuging the stationary phase cultures at 5000 rpm for 20 min. For detection and estimation of IAA content, cyanobacteria were harvested by centrifugation at 5000 rpm for 20 min. The culture filtrate was analysed for IAA production. 1.0 ml of supernatant was mixed with 2.0 ml of Salkowski's reagent (2.0 ml 0.5 M $FeCl_3$ in 98 ml 35% $HClO_4$) (Gordon and Weber 1951), and the intensity of pink color developed in the mixture after 30 min was quantified by a UV-Visible spectrophotometer at wavelength 530 nm. For standard, 1.0 mg of synthetic IAA (Hi media) was dissolved in 1.0 ml of methanol and used as stock. From stock solution, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 µl were taken, made up into 1000 µl with appropriate volume of distilled water and analysed as stated above. For single factor experiment, the effect of different concentration of tryptophan on IAA production was determined by incorporating 2.5, 5.0, 7.5 and 10.0 mg/ml tryptophan in culture media (Prasanna *et al*. 2010, Hussain and Hasnain 2011, Babu *et al.* 2013). The duration of incubation was determined by taking samples for IAA quantification after 10^{th}, 20^{th} and 30^{th} days of incubation (Prasanna *et al.* 2010, Hussain and Hasnain 2011).

To study the effect of IAA produced by cyanobacteria on plant growth, seed germination test was done using cowpea seeds. Healthy, bold seeds of cowpea were surface sterilised with 0.1% $HgCl_2$ for 5 to 7 min with continuous shaking. The seeds were then rinsed five times with sterile distilled water to remove any trace of $HgCl_2$. Surface sterilised seeds were divided into six groups of 15 seeds each. Two sets of seeds were maintained for each of the three treatments, viz. Absolute control: Seeds incubated in BG 11 medium without IAA; Positive control: Seeds incubated in BG 11 medium supplemented with synthetic IAA; and the Test: 5 ml culture supernatant of late log phase of cyanobacteria. Seed surface was dried and were distributed at a proper distance on sterile soft agar plates (0.5%) (Sachdev *et al.* 2009). Germination of cowpea seeds, incubated in the dark, was examined on sixth day, after which a 14-h photoperiod was maintained. The root and shoot lengths of the germinated seedlings were measured on the tenth day for determining the effect of IAA (Rae-Hyun and Hong-Gyu 2007).

To study the effect of IAA produced by cyanobacteria on germination of taro corm surface sterilised taro corms were exposed to 21 days late log phase culture filtrate of the IAA producing cyanobacteria. Corms were incubated in 0.5% water agar plates. Three treatments, an absolute control, a positive control and a test were maintained. The corms were incubated in dark for six days at 30°C. Percentage of germination, shoot length and root length were recorded.

A pot culture experiment was conducted in Completely Randomized Block Design (CRBD) to evaluate the effect of *Nostoc* on taro yield. The taro variety Muktakeshi and land race Telia were cultivated in grow bags each containing 10 kg sandy loam soil mixed with farmyard manure. There were three replications and each replication had 30 plants. Three fertilizer treatments were given to plants, viz. T_1: Recommended chemical fertilizer only; T_2: chemical fertilizer (Half of the recommended dose) + *Nostoc*; and T_3: *Nostoc* only. After sprouting of taro corms all the plants

were maintained under water-logged conditions with two centimetre level of water.

For RSM design and statistical analysis, based on the results of single factor experiment, levels of these independent variables, viz. tryptophan concentration (X_1), pH (X_2) and incubation time (X_3) were optimized for maximum IAA production by *Nostoc* using response surface methodology adopting the Box-Behnken statistical design (Box and Behnken 1960). The independent variables were coded at three levels (-1, 0, and 1). The factor and levels used for the experiment was: viz. tryptophan concentration (X_1) - 0.5 mg/ml (low), 2.5 mg/ml (medium) and 5 mg/ml (maximum); pH (X_2) - 5 (Low), 7 (medium) and 9 (maximum); and incubation time (days) (X_3) - 7 (low), 14(medium), 21 (maximum). The complete design consisted of 15 experimental points (Table 1) including three replications of the central points (all variables were coded as zero). A randomized experimental order was used to reduce the effect of unexplained variability on the observed response. Data were analyzed using ANOVA to determine the level of fitness and the effects of linear, quadratic, and interaction variables on IAA production by *Nostoc*. The data recorded in triplicate, unless specified, were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple range Test (DMRT) in accordance with the experimental design (CRBD) using the software R (version 3.2.1) and critical differences (CD) were calculated. RSM analysis was done using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

IAA production by cyanobacteria as influenced by the strain

In the present study, five nitrogen fixing filamentous cyanobacteria strains, viz *Anabaena* (CCC 660), *Aulosira* (CCC662), *Cylindrospermum* (CCC 665), *Nostoc* (CCC670) and *Tolypothrix* (CCC679) were screened for the production of growth regulator IAA. Among the five strains, *Aulosira* (CCC662), *Nostoc* (CCC670) and *Tolypothrix* (CCC679) produced considerable amount of IAA. The production of IAA by cyanobacteria was ascertained by Salkowski's reagent test. The IAA was quantified and found that *Nostoc* produced maximum amount of IAA (2.525 ± 0.01 µg/ml) after 15 days of culturing (Table 2). *Aulosira* produced 0.14 ± 0.07 µg/ml, whereas *Tolypothrix* produced 0.21 ± 0.01 µg/ml IAA. Therefore, *Nostoc* (CCC670), *Aulosira* (CCC662) and *Tolypothrix* (CCC679) were selected for further studies.

IAA production by cyanobacteria as influenced by culture period, tryptophan and pH

The time course of IAA production by cyanobacteria revealed maximum IAA production in *Aulosira* and *Tolypothrix* on 20th day of culturing (Table 3). On 20th day and 30th day of culturing IAA in the culture filtrate of *Aulosira* was 2.58 ± 0.36 µg/ml and 0.21 ± 0.01 µg/ml respectively. In *Nostoc* culture the IAA production was 3.62

Table 2 IAA production by the five cyanobacteria after 15 days culture period

Cyanobacteria	IAA released to the culture media (µg / ml)
Anabena	0.04
Aulosira	0.15
Cylindrospermum	0.06
Nostoc	2.53
Tolypothrix	0.21
Control (BG11 medium)	0.00
CD (P=0.05)	1.06

Table 1 The real time IAA production by *Nostoc* and predicted values according to Box-Behnken design

Run	Variables			Code values			IAA concentration (µg/ml)	
	Trptophan (mg/ml)	pH	Incubation time (Days)	X1	X2	X3	Experimental values	Predicted values
1	0.50	5	14	-1	-1	0	0.294	-0.7
2	0.50	9	14	-1	1	0	0.471	1.94
3	5.00	5	14	1	-1	0	10.140	11.74
4	5.00	9	14	1	1	0	14.542	14.42
5	2.75	5	7	0	-1	-1	2.121	3.93
6	2.75	5	21	0	-1	1	10.929	10.71
7	2.75	9	7	0	1	-1	6.025	6.61
8	2.75	9	21	0	1	1	13.170	13.39
9	0.50	7	7	-1	0	-1	0.822	0.01
10	5.00	7	7	1	0	-1	8.227	6.89
11	0.50	7	21	-1	0	1	0.798	1.18
12	5.00	7	21	1	0	1	19.419	19.28
13	2.75	7	14	0	0	0	7.402	8.66
14	2.75	7	14	0	0	0	10.618	8.66
15	2.75	7	14	0	0	0	10.374	8.66

Table 3 Optimisation of culture period for maximum IAA production by three cyanobacterial strains

Isolate	IAA produced (µg/ml)		
	10th day	20th day	30th day
Aulosira	0.21	2.58	0.21
Nostoc	0.24	3.62	8.73
Tolypothrix	0.10	1.11	0.09
CD (P=0.05)	0.02	0.98	1.19

± 0.94 µg/ml on 20th day which increased to 8.72 ±1.22 µg/ml on 30th day. The IAA produced by *Aulosira* (CCC662) was at par with that of *Tolypothrix* (CCC679), whereas IAA produced by *Nostoc* (CCC670) was significantly greater (P = 0.01) than that of *Aulosira* and *Tolypothrix*. The optimum concentration of tryptophan for maximum IAA production was also determined for the strains *Aulosira* and *Nostoc* (Fig 2 A). For both the strains 7.5 mg/ml of tryptophan was found to be the optimum.

Based on the single factor analysis the parameters tryptophan concentration, pH and incubation time for maximum IAA production by *Nostoc* were optimised by designing a RSM experiment to determine the influence of interacting factors on the outcome (Myers 1971, Box *et al.* 1987). The experimental and the predicted values of the RSM analysis were statistically analysed using ANOVA to check the suitability and fit of the design. The cardinal points of the response, IAA production, based on the selected three independent variables were extrapolated.

A second degree polynomial equation derived from multiple regression analysis explains the IAA production by *Nostoc* considering the significant factors.

IAA = 6.3171 + 2.26563 * TRP* + 0.67063 * pH – 0.00504 * CT* – 0.36019 * TRP * TRP + 0.178027 * TRP * CT

TRP = Tryptophan content per ml culture medium, CT= Culture period.

The amount of IAA produced by *Nostoc* varied between 2.90 µg and 19.42 µg per ml culture medium. Results of the RSM analysis represent the expected responses and correlation between variables. From the results, it is obvious that the selected parameters, viz. tryptophan content per ml culture medium (P = <0.0001), pH (P = 0.04) and culture period (P = 0.01) were significantly influencing IAA production by *Nostoc*.

The surface plot shows the effect of pH and tryptophan content per ml culture medium on IAA production by *Nostoc* at a fixed culture period of 14 days. The maximum IAA (12.48 µg/ml) was produced at 4.91 mg tryptophan content per ml culture medium at pH 6.21. The regression analysis showed that there is no significant correlation between pH and tryptophan content per ml culture medium (P = 0.199). The effect of culture period and tryptophan content per ml culture medium on IAA production by *Nostoc* at a fixed pH 7.00 was studied. The maximum IAA production (17.88 µg/ml) was recorded at 20th day of culturing and 4.55 mg tryptophan content per ml. The regression analysis showed significant correlation between culture time and tryptophan content per ml culture medium (P = 0.011). The effect of culture period and pH on IAA production by *Nostoc* at a fixed tryptophan content per ml culture medium (2.75 mg/ml) was extrapolated. The maximum IAA production (11.54 µg/ml) was recorded at culture period of 20th day at pH 7.11. The P value of the interaction study of these two factors was 0.58 and was not significant. The model F value was 25.89 and P value was 0.001. The coefficient of determination (R^2) of the experimental model was 97.90% and that of the predicted model was 95.75% which indicate that the experimental value of IAA production nearly matched with the model-predicted values (Table 1). Based on the regression equation the optimum values for the selected variables for maximum IAA production by *Nostoc* was at 2.75 mg tryptophan per ml culture medium, pH 7.0 and culture period of 14 days. The ANOVA results substantiated that all the selected variables are significant in determining IAA production by *Nostoc*. This is in agreement with Lee *et al.* (2010), Lin and Pan (2015) and Li *et al*. (2015).

The effect of cyanobacteria produced IAA on the sprouting of taro corm

The results of the effect of *Nostoc* produced IAA on taro corm sprouting indicate that taro corms exposed to BG11 medium and synthetic IAA had 33% sprouting, whereas corms exposed to *Nostoc* culture filtrate had 100% germination. The biometric parameters such as root length and shoot length of taro plants emerging from corms exposed to *Nostoc* culture filtrate were significantly greater than that of control and synthetic IAA treatment. The average shoot and root length of taro plants emerging from corm exposed to *Nostoc* culture filtrate was 1.85 cm and 2.87 cm respectively compared to synthetic IAA which resulted in an average root and shoot length of 1.73 cm and 1.95 cm respectively.

The effect of cyanobacteria produced IAA on the germination of cowpea seeds

The effect of *Nostoc* produced IAA was ascertained on germination of cowpea seeds (Table 4). In the case of cowspea seeds, the germination was 33.30% in untreated control and that of seeds supplemented with 30 µg/ ml synthetic IAA and *Nostoc* culture filtrate containing IAA

Table 4 Effect of culture supernatant of *Nostoc* on the germination and biometric parameters of cowpea

Treatment	Average shoot length (cm)	Average root length (cm)	Germination (%)	
			(30 µg/ml IAA)	(100 µg/ml IAA)
Control (BG11)	20.50	11.40	33.30	36.60
Synthetic IAA	16.40	8.80	43.30	73.33
Nostoc culture supernatant	18.20	15.20	46.60	80.00

was 43.33% and 46.63% respectively. With an increase in IAA content from 30 µg/ml to 100 µg/ml, the germination percentage of cowpea seeds exposed to synthetic IAA and *Nostoc* culture filtrate containing IAA increased to 73.33% and 80% respectively. Cowpea seeds treated with *Nostoc* culture filtrate had greater percentage of germination than synthetic IAA. The average shoot and root length of cowpea seedlings emerging from seeds exposed to *Nostoc* culture filtrate was 15.2 cm and 18.2 cm respectively whereas that of synthetic IAA was 8.8 cm and 16.4 cm respectively.

The effect of cyanobacteria produced IAA on taro corm yield

The pot culture experiment revealed that the taro land race Telia and variety Muktakesi responded significantly to *Nostoc* inoculation. Corm yield of land race Telia was 158 g/plant in which recommended dose of chemical fertilised was used. The corm yield increased by 32.91% (210 kg/plant) where *Nostoc* was used instead of chemical fertilizer. Compared to the land race Telia the variety Muktakesi responded better to *Nostoc* inoculation. In the case of variety Muktakesi the mean corm yield was 180 g/plant where chemical fertilizer was applied, whereas 280 g corm per plant was obtained in *Nostoc* inoculated pots. Application of chemical fertilizer along with *Nostoc* resulted in greater yield in the variety Muktakesi (320 g/plant).

IAA is a multi functional growth regulator which can promote plant growth, protect plants from pathogens and play a major role in plant signalling pathway (Spaepen *et al.* 2007). In the present study, IAA production at 15th day significantly varied between 0.040 and 2.525 µg/ml culture medium among five nitrogen fixing filamentous cyanobacteria strains, viz *Anabaena* (CCC 660), *Aulosira* (CCC662), *Cylindrospermum* (CCC 665), *Nostoc* (CCC670) and *Tolypothrix* (CCC679). This is in agreement with IAA production reported in a wide array of microorganisms. Rhizospheric and free living cyanobacteria produce growth regulators such as cytokine and IAA (Sergeeva *et al.* 2002, Mohite 2013, Shariatmadari *et al*. 2013). Sergeeva *et al.* (2002) screened 34 free living and symbiotically competent cyanobacteria and found that 38% of the free living and 83% of the symbiotic isolates produced auxin like substances, as IAA-methyl trimethylsilyl ester (peaks at m/z 202.1050) and [$^{13}C_6$] IAA-methyl trimethylsilyl ester (peaks at m/z 208.1250). Babu *et al.* (2013) also reported the production of auxin like compounds by cyanobacteria *Leptolyngbya* sp. and *Geitlerinema* sp. Hussain and Hasnain (2011) reported growth regulators in *Synechocystis* sp. and *Chroococcidiopsis* sp. culture media in terms of pmol mg^{-1}. We also found that the IAA production significantly increased between 10 th and 20/30th day of culture period in three of the cyanobacterial strains, viz. *Aulosira*, *Nostoc* and *Tolypothrix.*

In the present study, there was an absolute requirement of tryptophan for IAA production by the selected strains, *viz. Aulosira, Nostoc* and *Tolypothrix*. The cyanobacteria strains were inoculated into nitrogen free BG11 medium without tryptophan and found that IAA production was negligible or nil. Although nitrogen ($NaNO_3$- 1.5g/L) was supplemented to the medium and incubated without tryptophan the strains failed to produce IAA. Cyanobacteria are capable of converting exogenous tryptophan to IAA and tryptophan inhibited heterocyst formation and nitrogen fixation by cyanobacteria (Sergeeva *et al*. 2002). During the initial stages of growth, tryptophan can act as a nitrogen source and in the later stages of growth it will be converted to IAA.

Tryptophan was reported to be a main precursor of IAA synthesis in many bacteria like *Azospirillum*, *Pseudomonas putida* and *Rhizobium*. Supplementing culture media with tryptophan induced greater IAA production by these bacteria (Theunis *et al.* 2004). Srinivas *et al.* (2002) demonstrated that in anoxigenic phototrophic bacteria tryptophan and light can significantly enhance IAA production. According to Spaepen *et al.* (2007), tryptophan is the main precursor of IAA production in bacteria and the five different pathways - Indole-3-acetamide pathway, Indole-3-pyruvate pathway, Tryptamine pathway, Tryptophan side-chain oxidase pathway and Indole-3-acetonitrile utilise tryptophan as precursor for IAA production. Trp-dependent synthesis of auxins for plant growth promotion was also reported in the PGPR *B. amyloliquefaciens* FZB42 (Idris *et al.* 2007). The nitrogen fixing bacteria *Pseudomonas* and *Azotobacter* isolated from the root and rhizosphere samples of sugarcane required tryptophan as a precursor for IAA production (Ashraf *et al*. 2011). In their study, they used malate (nitrogen free) medium supplemented with 100 mg/L L- tryptophan for IAA production, whereas none among the twelve nitrogen fixing bacterial strains produced IAA in the absence of tryptophan. Although tryptophan is necessary for IAA production, in the present study IAA production was inhibited at higher concentration of tryptophan. This is in agreement with the results of Hussain and Hasnain (2011). They reported that the optimum tryptophan concentration for the isolate *Phormidium* SM-14 was 500 µg/ml and that of SM-15 was 400 µg/ml and tryptophan at very high concentrations inhibited auxin production. Conversely, Prasanna *et al.* (2010) reported that tryptophan is not necessary for IAA production by two strains of *Anabaena,* viz. CW1 and RP 9 but a light: dark cycle is necessary for IAA production in cyanobacteria but a continuous illumination or complete dark condition is not.

In the present study, sprouting of taro corm was induced by treating taro corms with *Nostoc* culture filtrate. The induction effect of *Nostoc* culture filtrate was ascertained on germination of cowpea seeds. Cowpea seeds treated with *Nostoc* culture filtrate had 13.33% greater germination than untreated seeds. Several authors demonstrated a significant increase in seed germination of crop plants such as wheat, sorghum, maize, lentil and sugar beet as a result of the treatment with the culture filtrate or cell extract of cyanobacteria (Kumar and Kaur 2014, Essa *et al*. 2015). Cyanobacteria culture filtrate induced

germination of seed in rice (Prasanna et al. 2010), wheat (Hussain and Hasnain 2011, Mazhar and Hasnain 2011) and maize, tobacco (Boopathi *et al*. 2013). The seed germination of *Sorghum durra* was markedly promoted with *Anabaena oryzae* and *Synechococcus* sp. of cyanobacteria (Essa *et al.* 2015). Similarly, the culture filtrate of a mangrove root associated cyanobacterium *Phormidium* sp. MI405019, capable of producing IAA, increased seed germination of tobacco by 40% and induced multiple roots from tobacco callus (Boopathi *et al*. 2013). Application of *B. subtilis*, a bacteria isolated from cow dung, suspension on the surface of cut tubers of white yam (*Dioscorea rotundata*) increased the number of sprouts over the untreated tubers (Ray and Nedunchezhiyan 2016). To the best of our knowledge, ours is the first report of cyanobacteria culture filtrate containing IAA inducing sprouting of a tuber crop like taro.

IAA produced by cyanobacteria is capable of stimulating plant growth. The cyanobacterial IAA was positively correlated with the root growth parameters, shoot length, spike length and weight of seeds in wheat. Hence, the cyanobacteria could be used as a potential biofertilizer for enhancing growth of wheat plant (Hussain and Hasnain 2011). The length and biomass of shoot and root of *Sorghum durra* seedling was significantly enhanced by *Anabaena oryzae* and *Synechococcus* sp. of cyanobacteria (Essa *et al*. 2015). Hussain and Hasnain (2011) and Mazhar *et al*. (2013) reported an enhancement in shoot length, number of lateral roots of the wheat plants treated with cyanobacterial strains. Application of dried cyanobacteria biofertilizer significantly increased leaf length, weight of fresh and dry leaf, leaf number per plant, leaf area and dry root biomass of lettuce over the control (Hashtroudi *et al*. 2013, Menamo and Wolde 2013). The plant growth promoting properties of Rhizobacteria has been reported (Ahemad and Khan 2010, Mohite 2013). Application of *B. subtilis* suspension on the surface of cut tubers of white yam increased the length and weight of root and shoot and root:shoot ratio over the untreated tubers (Ray and Nedunchezhiyan 2016). The greater shoot and root length and corm yield of taro due to *Nostoc* in pot culture also supports these findings.

The significance of our finding is that since the tuber crop selected for this study, taro is well adapted to waterlogged conditions, the diazotrophic cyanobacteria can be used as a biofertilizer for this crop.

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