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Research Article

EFFECT OF PYRROLOQUINOLINE-QUINONE ON GERMINATION AND GROWTH CHARACTERISTICS OF GROUNDNUT (*ARACHIS HYPOGAEA*)

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Abstract: Pyrroloquinoline-quinone (PQQ) has been implicated as a plant growth regulator in several studies. However, a detailed mode of action of the molecule is yet to be described in plants. It is known to be an antioxidant and a redox cofactor. Natural and nature identical molecules that improve the seed germination and plant growth are helpful in designing novel organic inputs. In order to assess the usability of PQQ to design such formulations, we studied the effect of various concentrations of synthetic PQQ on the seed germination and growth characteristics of groundnut. We found that in groundnut, PQQ treatment shows significant difference in root development. Remarkably, the seedlings arising from 1.00 mM PQQ treated seeds showed triple the fresh weight of roots as compared to the controls. There was also an improvement in seed germination and other growth characteristics in the PQQ treated seeds. Our results show that PQQ is a promising molecule in designing novel formulations for plant growth and development.

Keywords: *Pyrroloquinoline Quinone [PQQ], Groundnut [Arachis Hypogaea], Seed Germination, Growth Characteristics, Lateral Roots*

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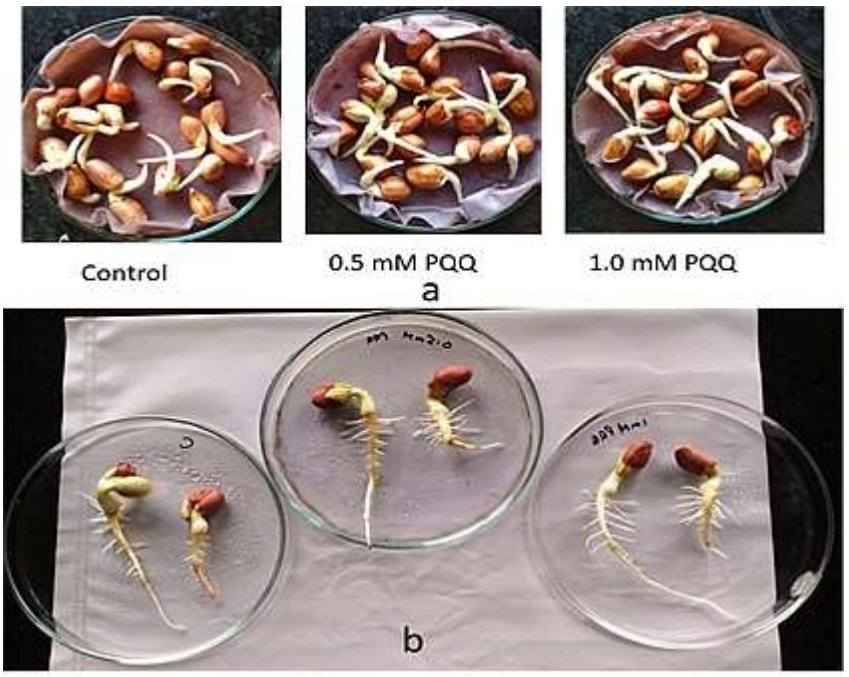
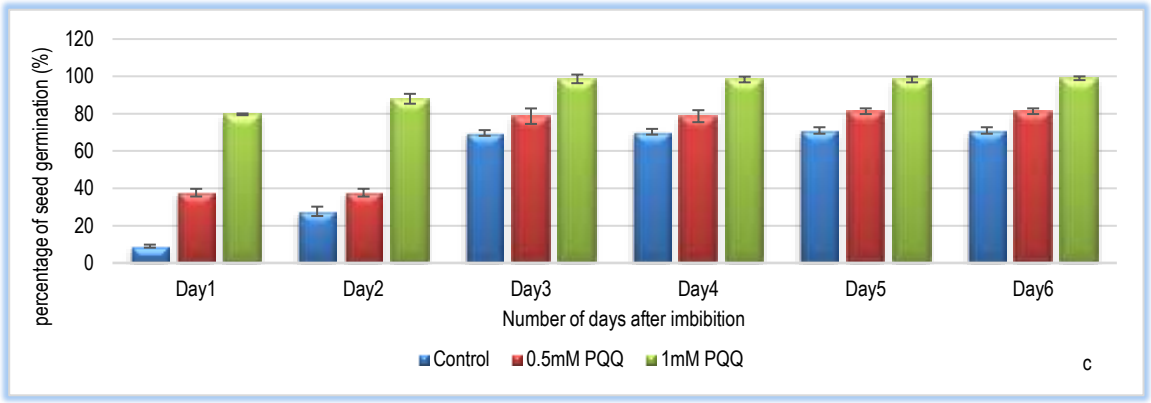
Introduction

Bio-stimulators are regarded as a sub-group of bio-regulators, and exhibit an important effect on the regulation of life processes within plants in various ways [1]. They are extracts of natural origin, i.e., derived from plant, animal or microbe. The role of certain regulatory molecules such as Pyrroloquinoline quinone (PQQ) has been studied extensively in relation to animal and human functional aspects with comparatively few citations available in relation to its functional aspects in plant development [2]. Several such molecules that have well-defined function in animals but not in plants in-spite of their presence in plants are waiting to be functionally characterized [3]. Pyrroloquinoline-quinone (PQQ) was first identified in methylotrophic bacteria [4] as a coenzyme for methanol dehydrogenase, and named as methoxatin. PQQ is an antioxidant and a redox cofactor, found in plant and animal tissues in the nanogram-to-gram range even though plants and animals do not produce PQQ themselves [5]. PQQ is water soluble, heat stable, and has the ability to carry out redox cycles [6]. Phosphate solubilization by rhizobacteria is mainly carried out by production of Gluconic acid or enzymes such as acid phosphatase. The enzyme that plays the main role in biosynthesis of Glucuronic acid is glucose dehydrogenase, which requires the cofactor PQQ to carry out its activity [7]. A role of PQQ in bio-control activity is evident from the study where the PQQ minus cells of *Roseateles aquatilis* become ineffective in their bio-control activity against *Agrobacterium vitis*, while the bio-control potential is restored on expression of complete operon of PQQ synthesis [8]. The significant increase in fresh weight of cucumber seedlings on being treated with various concentrations of synthetic PQQ [2], suggests a great potential for this molecule in crop productivity. The influence of climate change and anthropogenic factors on environment has directly affected soil condition and hence, has a major bearing on crop productivity, and also the surrounding rhizobacteria. The growing awareness about hazards of chemical and inorganic inputs in agriculture has brought bio-fertilizers, bio-pesticides and plant growth regulatory molecules of natural/nature identical origin into limelight. This shift towards sustainable agriculture practices is focused on yield increase and remediation of dwindling soil nutrients. Detailed study of molecules that are non-toxic, natural, and have positive effect on growth of plants that are growing under sub-optimal conditions, will help in designing the most effective methods for their application. In the present short communication, we studied the effect of imbibing the seeds of groundnut [*Arachis hypogeal*] in different concentrations of aqueous solutions of synthetic PQQ. The most significant difference in imbibed and unimbibed seeds was the growth of lateral roots. The imbibed seeds showed profuse growth of lateral roots within a couple of days of germination. The seeds were then shifted to pots and the growth parameters were observed. An overall improvement in germination and growth parameters was also observed in imbibed seeds. The root system, which is the main body responsible for nutrient absorption, anchorage and food storage, consists of primary roots, lateral roots and root hairs. The primary root originates from the radical and particularly in dicots and gymnosperms which consist of a tap root system, grows downwards into the soil while anchoring the plant. The secondary or lateral roots grow horizontally or diagonally from the primary root [9]. These serve to increase the anchorage and absorption capacity, and soil area coverage of the roots. Hairy roots exhibit plagiotropism, abundant mass, extensive lateral branching, vigor, and fast hormone-independent growth [10]. Roots of legumes such as groundnut and soybean establish symbiotic relationship with nodule forming, nitrogen fixing bacteria such as *Rhizobium sps*. In most legumes such as soybean, a root hair entry for rhizobium infection has been suggested [11], while in others such as groundnut the infection occurs via cracks. In groundnut the root nodules develop where the epidermis and cortex of the parent root are broken due to the emergence of lateral root [12]. This suggests that higher number of lateral roots provides better opportunity for colonization of rhizobia, which would lead to increase in nitrogen fixation and hence better crop productivity. A well-developed root system is also helpful in increasing moisture retention and stress tolerance.





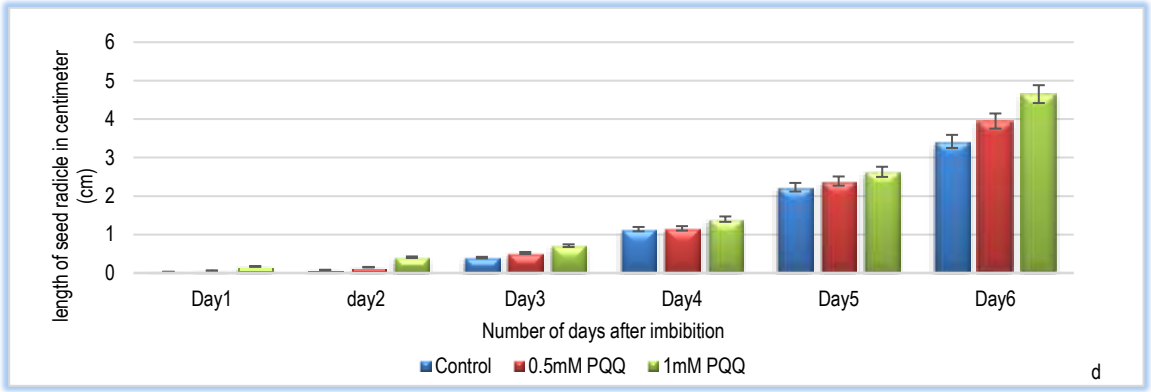


Fig-1 Seed germination and radical growth in Pqq treated and control groundnut seeds [a] Number of seeds germinated after 3 DAI where 1.0 mM PQQ treated seeds showed 100% germination and longer radicles as compared to Controls. [b] Lateral root growth in PQQ treated and control seeds at 6 days after germination. [c] Average percent of seeds [treated and control] germinated through a period of 6 days [d] Average seed radical lengths [cm] [in treated and control seeds] through a period of 6 days.

Table-1 *Growth characteristics of groundnut plants grown from PQQ treated [0.5mM and 1.00mM] and control seeds*

	Average Root length in cm	Average Shoot length in cm	Average Root Weight in gm		Average Shoot Weight in gm		Average number of leaves	Average Area of leaves in Sq cm
			Fresh weight	Dry Weight	Fresh Weight	Dry Weight		
Control	9.93±1.48	27.72±4.99	0.21±0.01	0.053±0.003	2.82±0.02	0.45±0.08	33.15±0.02	46.21±7.98
0.5mM PQQ	11.6±2.23	31.13±6.20	0.35±0.03	0.086±0.002	3.42±0.02	0.59±0.00	33.18±0.01	71.77±9.10
1mM PQQ	17.43±2.76	31.29±4.03	0.59±0.02	0.086±0.034	3.62±0.02	0.61±0.01	36.86±0.02	57.73±4.31

Fig-2 Plants growth promotion with PQQ treatment [a] Seedlings from PQQ treated seeds showed a profuse growth of lateral roots as compared to controls at day 7 after potting. [b] Plants arising from PQQ treated seeds are more robust compared to control plants [c] PQQ treatment has significant effect on root hair growth of groundnut plant

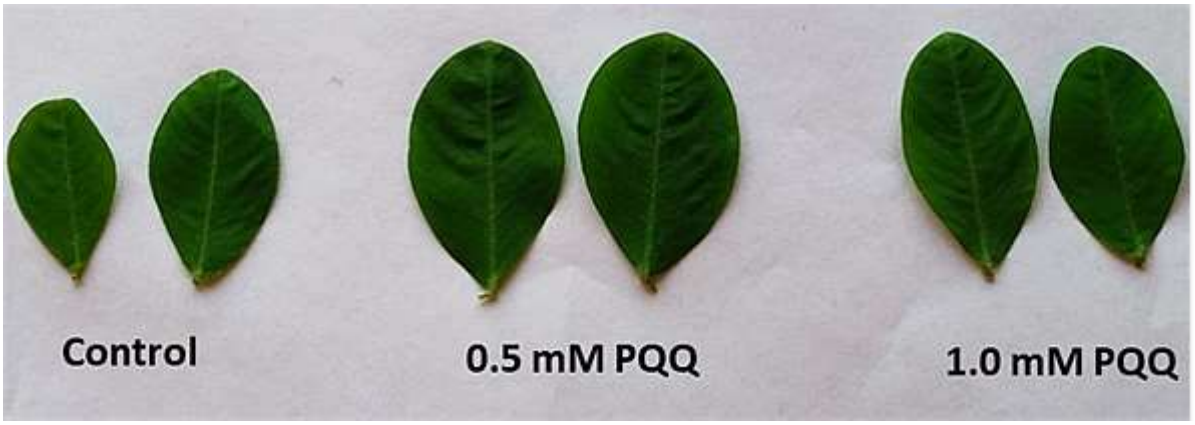


Fig-3 PQQ treatment increases the growth of leaf area in groundnut

Materials and Methods

Germination experiments

All the germination and growth parameter testing experiments were carried out in triplicates with same batch of seeds. Wild type organic groundnut seeds were collected from the Rangwasa farm owned by Indore Biotech Inputs and Research and homogenized. The seeds were surface sterilized with 100% ethanol in an Erlenmeyer flask and were treated with 1% Na hypochlorite for 2 min followed by six times washing with sterile water. A sample of Pyrroloquinoline quinone bis sulphate was obtained from Anthem Biosciences Pvt. Ltd, India. The seeds were soaked overnight in either 0.5 mM PQQ solution, 1mM PQQ solution or De-ionized (DI) water water. Seeds soaked in DI water were treated as control. Twenty five seeds from each treatment (imbibed and controls) were put in sterilized Petri dishes containing filter paper (Whatman #2) and the Petri dishes were kept in an incubator at 30ºC until radicle emergence. Radicle emergence test and speed of germination were determined as has been described earlier [13].

Plant growth promotion studies

At 6 days after completion of germination, the seeds were transferred to soil and the growth characteristics were observed. The root and shoot lengths were monitored at regular intervals. At 30 days after transfer, plants were uprooted and the fresh weight and dry weight of roots and shoots were recorded. Also, the average number of leaves and average area of leaves were recorded. Average area of leaves was recorded using the android based Petiole app (www.petioleapp.com).

Results

PQQ improves germination rate and percentage in groundnut seed

PQQ has been reported to have a positive effect on plant growth. In order to see its effect on germination of groundnut seeds, we imbibed the seeds of groundnut with two different concentrations of PQQ (0.5mM and 1mM). Control seeds were imbibed with DI water. Interestingly, around 80% of the seeds imbibed with 1.0 mM PQQ showed radicle emergence on 1st day after imbibition (DAI). This number was about 40% in seeds imbibed with 0.5mM PQQ and 10% in control. By 3rd DAI, these numbers had gone upto 100% in 1.0mM PQQ, 80% in 0.5mM PQQ and 70% in control treatments [Fig-1a,c]. It was observed that the average radical lengths of the germinated seeds were longer in PQQ treated seeds as compared to control seeds and also the emergence of lateral roots was faster in PQQ treated seeds, *i.e*, lateral roots were visible on the radicles of PQQ treated (both 0.5mM and 1.0 mM) seeds by day 5 after germination, while lateral root emergence in control seed radicles was observed on day 6 [Fig-1 b,d].

PQQ improves lateral root growth in groundnuts seedlings

The germinated seeds were transferred to soil, in separate plastic bags, 6 days after germination was complete and the growth parameters were monitored at regular intervals. 10 seedlings were uprooted from each treatment one week after the transfer. Average lengths of seedlings were found to be 25cms in 1.0 mM PQQ, 19 cms in 0.5mM PQQ and 17cms in control treatments. At this stage, a significant difference was observed in the roots of seedlings arising from PQQ treated seeds. The seedlings from PQQ treated seeds showed a profuse growth of lateral roots which was higher than in control seedlings [Fig-2a]. An overall improvement in growth parameters was observed in PQQ treated plants [Fig-2b]. All the plants were uprooted after 30 days and fresh and dry weights of roots and shoots were recorded. The average weight of fresh root was found to be 0.59±0.02 g in 1.0mM PQQ, 0.35±0.03 g in 0.5mM PQQ, and 0.21±0.01 g in control treatments. The average fresh weight of roots of plants grown from 1.0 mM PQQ treated seeds showed approximately triple the fresh weight of controls while the average weight of dry roots of plants grown from 1.0 mM PQQ treated seeds showed approximately double the dry weight of controls. At the same time the average root length of the seedlings from 1 mM PQQ treated seeds was approximately double that of controls [Table-1]. Interestingly, there was also a difference in the average number of leaves [Table-1] and leaf area in the plants grown from PQQ treated seeds [Fig-3], [Table-1] as compared to controls; while there was not much difference in the chlorophyll content of the leaves as studied using spectrophotometer analysis.

Discussion

Pyrroloquinoline quinone has been described as a plant growth regulator in several reports. In our experiments we found that imbibition's of different concentrations of PQQ by groundnut seeds not only affected the germination rate and percentage, but also had positive effect on the growth and development of plants arising from the treated seeds. Further studies on development, stress tolerance and yield improvement, which were not in the scope of this study, are required to fully understand the actual benefit of PQQ treatment in the field. However, this study is definitely an indicator of the potential of PQQ in improving growth and development of groundnut plants. Most significant difference was observed in the growth of roots. The profuse growth of lateral roots in the plants arising from the 1.0 mM PQQ treated seeds was evident from the seedling stage and later showed triple the fresh weight of those from controls and double the dry weight of those from controls. This difference between fresh weight and dry weight can be attributed to the moisture holding capacity of the roots. Enhanced lateral root growth has several benefits especially for plants like groundnut where the rhizobium infection starts at the junction between parent root and the lateral root. Hence enhanced growth of lateral roots ensures higher surface area for root nodule formation and better nitrogen fixation, which ultimately affects the yield in a positive way. A well-developed lateral root system also improves the soil area coverage by the roots thus ensuring improved nutrient uptake and better water holding capacity. This renders a plant stress resistant especially in drought prone soils. More such studies of the effect of PQQ on various plant species and at different stages of plant growth and development would help in designing the most effective method of its applications.

Conclusion

In present investigation, synthetic Pyrroloquinoline- quinone was found to improve seed germination and seedling growth parameters in groundnut.

Pyrroloquinoline quinone has earlier been implicated in many studies as a plant growth regulator although a detailed functional characterization is yet to be carried out. We conclude that natural and nature identical molecules such as PQQ have immense potential in organic product development and therefore a detailed study of their effect on various growth aspects of various plant species, and also in soil application, would be very helpful in designing new formulations of organic inputs.

Application of research: The research shows that Pyrroloquinoline quinone which is implicated as plant growth regulator in several earlier studies also helps in improving germination and growth characteristic, particularly lateral root growth, in groundnut. This indicates the usability of PQQ in formulation of various organic inputs for plant germination and growth.

Research category: Seed germination

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Research project name or number: Research station trials

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Rangwasa Farm, Madhya Pradesh

Cultivar / Variety / Breed name: Groundnut (*Arachis hypogaea*)-Wild type

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Herve J. J. (1994) *Comptes Rendus Acad. Agric. Fr.* 80, 91-102.
[2] Choi O., Kim J., Kim J., Jeong Y., Moon J., Seuk Park C., Hwang I. (2008) *Plant Physiol*., 146,657–668
[3] Ratnaparkhe S.M., Egertsdotter E.M.U., Flinn B.S. (2009) *Planta,* 230, 339-354
[4] Salisbury S.A., Forrest H.S., Cruse W.B.T., Kennard O. and Westerling *et al*. (1979) *Nature,* 280, 843–844
[5] Kumazawa T., Seno H., Urakami T., Matsumoto T., Suzuki O. (1992) *Biochim Biophys Acta,* 1156,62–6.
[6] Stites T.E., Mitchell A.E. and Rucker R.B. (2000) *J.Nutr*.,130 719–727
[7] Goldstein A.H. (1986) *Am. J. Alternat. Agric*, 157–65
[8] Guo Y.B., Li J., Li L., Chen F., Wu W., Wang J. and Wang H. (2009) *Appl. Environ. Microbiol*, 75, 6792–803
[9] Wu J.,Guo Y. (2014) *Annals of Botany,* 114, 841–851.
[10] Guimaraes L.A., Mota A.P.Z., Araujo A.C.G., de Alencar Figueiredo L.F., Pereira B.M., de Passos Saraiva M.A. (2017) *Plant Mol Biol*, 1–2, 79-96.
[11] Laurent Brechenmacher, Zhentian Lei, Marc Libault, Seth Findley, Masayuki Sugawara, Michael J. Sadowsky, Lloyd W. Sumner (2010) *Plant Physiology*, 153(4), 1808-1822
[12] Ryosuke Tajima, Junabe O. New Lee, Shigenori Morita and Alexander lux. (2008) *Annals of Botany*, 101, 491-499.
[13] Asghar Farajollahi, Bahram Gholinejad, and Hamed Jonaidi Jafari (2014) *Hindawi Article ID* 245686, 5.