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Rapid Detection of Citrus Greening (Haunlongbing) by Iodine Kit Method and its Validation using Polymerase Chain Reaction (PCR)

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A B S T R A C T

Citrus greening (HLB) is deadly diseases caused due to gram negative bacterium called α-probacteria. The transmission of disease occurs by a vector psyllid (*Diaphori citri*). It cannot be controlled fully by chemical means only the production of diseases free panting material and removal of infected part are the measures to overcome this disease. The early detection of this disease helps to keep the orchards free of diseases and losses. On the basis of visual symptoms the diagnosis of HLB under field condition is very difficult. The symptoms occurred due to HLB are like blotchy mottle pattern of leaves and yellowing of leaves which can also be the reason of nutrient deficiency like zinc, manganese and iron. So, to avoid this confusion present study was designed to produce rapid diagnostic test for the disease. The Iodine kit method was standardized for the detection of disease in field with accuracy and rapidity. The HLB infected leaf produce high starch compare to healthy leaf because of that there is production of brown colour on reaction with iodine while yellow colour was observed in healthy leaves this shows the absence of HLB infection. Later the polymerase chain reaction (PCR) was used to confirm and validate the result obtain by iodine kit method using specific primer to 16S rDNA region of Indian '*Ca*. L. asiaticus' i.e. OI1/OI2c species. The amplification was observed in HLB infected leaf at 60^0c with amplicon size of 1160 bp, which was absent in healthy leaf sample. Thus, the iodine kit method has great potential to provide an improved, cost effective, rapid, user friendly and in situ method for diagnosis of *Ca. L. asiaticus'* for the farmer, nurseryman, mobile plant pathology laboratories, bud wood certification programme and quarantine programme and in offices. The present methods of disease diagnosis helps in the reduction of disease outbreak and keep the orchard free from diseases by helping us in the screening of disease free planning material.

Introduction

Citrus is one of the most valuable and widely grown fruits in the world which provides nutrition to the local people and is used as an indispensible cash crop. But now a days Asian citrus plantation have brought serious yield loss and deterioration of fruit quality due to diseases associated with it (Whiteside, 1988). Citrus greening (Haunlongbing) is one of the devasting diseases which cause severe loss in citrus yield. There is 30-100% loss occur in citrus yield due to citrus greening infection globally (Ghosh and Das, 2012) while vidharbha region of maharashtra 8-43% of sweet orange and 6% Nagpur mandarin were infected by greening. It is characterized by chlorosis of leaves on one or more limbs (Fig 1 a), followed by twig dieback, sparse foliage, distinct yellow shoots, and fruits which do not fully colour at stylar end and remain green hence the name greening (fig 1b). Later the trees may show an open, spare foliage, severe fruit drop and many small yellow shoots in many cases result into severe decline and death (Roistacher, 1991).

The greening is caused by a gram negative bacterium called α-probacteria. It is widely distributed and serious disease in India transmitted by psyllid vector shown in fig 1 c (*Diaphoria citri*) and vegatative propagation (Ghosh, 2012). They are classified based on geographic location and sensitivity to temperature into three species: '*Ca L. asiaticus*' '*Ca. L. africanus*' and '*Ca.L. americans'* (Bove 2006; Jagoueix *et al.*,1997). On the basis of visual symptoms the diagnosis of HLB under field condition is very difficult.

The symptoms like blothchy mottle pattern of leaves and yellowing of leaves can also be the reason of nutrient deficiency like zinc, manganese and iron. So, to avoid this confusion a rapid diagnostic test (Iodine kit method) used for diagnosis of disease. It works on the principle of starch production.

The infected leaves produce six times more starch than healthy leaves. Recently number of researcher used this technique for diagnosis of HLB. It is not hundred percent sure techniques for diagnosis of citrus greening so to make it more accurate the results were validated using improved technology i.e. polymerase chain reaction (PCR).

The polymerase chain reaction (PCR) based on molecular techniques are powerful methods which has greatly facilitated detection of plant pathogen that otherwise would have been difficult or time consuming to detect using conventional technique (Dilip Ghosh and Das, 2012). Citrus being a predominantly vegetatively propagated crop, presence of pathogen in mother plants in nursery.

Since no chemical method is effective against this graft transmissible pathogen establishment of pathogen free nursery system is the only way to control this disease. This increases the necessity of conducting various experiments to detect the pathogen in citrus sample. So, the present study reports an effective Iodine kit method and its validation by PCR for faster and reliable detection of greening pathogen (*CLa*) in citrus cultivar and its usefulness to implement citrus bud wood certification programme in India.

Materials and Methods

The HLB infected leaves and healthy leaves of sweet orange (*Citrus sinesis*) were collected (Fig 2) from College of Horticulture, Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola. The mature citrus leaves showing symptoms were collected in a polythene bag. The upper surface of the infected leaf was scratched about 40 times with a piece of sandpaper.

The sand-paper piece harbouring tissue debris was then placed in a small polythene bag with a sealing mouth and 1 ml of pure water added in it. The sand-paper piece was rubbed in water thoroughly for washing of the tissue debris into the water. After that, a drop of iodine solution was added into the suspension in the bag and the solution was mixed by shaking. At last, the solution was observed for colour-change.

Extraction of DNA from infected leaf sample

DNA isolation method for detection of HLB was optimized as per standard protocol given by Dellaporta *et al.,* (1983). The quality of DNA obtained after extraction was confirmed by running it on 0.8% agarose gel (containing ethidium bromide @0.5 µg/ml) in a horizontal gel electrophoresis system.

Primers and polymerase chain reaction (PCR) for citrus greening

The PCR was carried out using citrus greening specific primers (OI1/OI2c) given in Table 1. The primers were designed on the basis of the sequence information reported and which is conserved among Asian strains of the greening bacterium (Jagoueix *et al.,* 1994). The PCR was carried out by using following parameters: one cycle at 94^0c for 3 min, 35 cycles at 94^0c for 1 min, 55-60^0c for 1 min and 72^0c for 45 sec followed by one cycle at 72^0c for 10 min. The PCR products were analysed by electrophoresis using 1% agarose gel and visualized in gel documentation unit.

Results and Discussion

Detection of citrus greening (HLB) using iodine kit

The test was carried out to distinguish between confusing nutrient deficiency symptoms and leaves that may be HLB positive and also helpful to select the best sample for PCR analysis thus helping to reduce the number of negative samples.

The iodine kit detection was carried out by collecting healthy and citrus greening (HLB) infected leaf samples of sweet orange. The piece of sandpaper was scratched on both HLB infected and healthy leaf sample and this scratched sandpaper was put into zip lock polyethylene bag so that after adding a drop of iodine solution into the zip lock bag, it can be rubbed properly to loose the leaf tissue debris which reacts with iodine solution. After adding iodine solution it was observed that there was a development of black colour in HLB infected leaf sample (fig 3)

This was due to the reaction of accumulated starch in the HLB infected leaves while the development of yellow colour in the healthy leaf sample showed no starch accumulation ultimately showed the absence of HLB infection to the healthy leaf plant shown in plate 1. A similar test was carried out by Hong and Truc (2003) on citrus species and they got similar results. This showed that the test was carried out successfully.

Validation of iodine kit method using polymerase chain reaction (PCR)

The results of iodine kit method were validated using polymerase chain reaction (PCR). The DNA isolated by CTAB method was confirmed on 0.8 percent agarose gel and quantified using a spectrophotometer. The DNA was treated with ribonuclease-A for RNA to cut off. Then pure quality DNA was used for PCR amplification with 50 ng/µl concentration. PCR was carried out using OI1 /OI2c primers specific to 16S r DNA region of α-probacteria and the amplified products were run on 1% agarose gel. The OI1 /OI2c amplify amplicon size 1160 bp in the infected leaf sample and no amplification was observed in healthy leaf as well as negative control sample shown in Fig 4.

Ghosh and Das (2012) while working on HLB detection in different citrus cultivars used similar OI1/OI2c primers and got similar band size *i.e.* 1160 bp for greening infected mosambi, Nagpur mandarin and acid lime plant while no band in a healthy plant.

Ruangwong and Akarapisan (2006) reported that a polymerase chain reaction (PCR) with specific primers OI1/OI2c used for detection of HLB bacterium produced specific band of 1160 bp for diseased leaves whereas no product from healthy citrus plants.

Table.1 Primer used for detection of citrus greening (HLB)

Primer	Nucleotide sequence	Amplicon size
FP (OI1/OI2C)	GCGCGTATGCAATACGAGCGGCA	1160 BP
RP (OI1/OI2C)	GCCTCGCGACTTCGCAACCCAT	

Fig.1 a) Chlorosis of leaves symptoms due to citrus greening infection, b) Greening of fruits symptoms induced by citrus greening c) Citrus Psyllid, *Diaphori citri* feeding on citrus plant

a b c

Fig.2 a, b) HLB infected leaf sample of sweet orange (*Citrus sinesis*), c) Healthy leaf sample of sweet orange (*Citrus sinesis*)

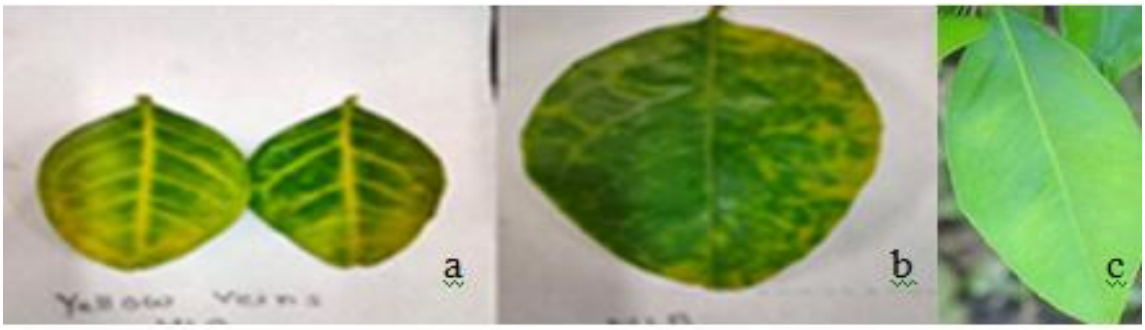
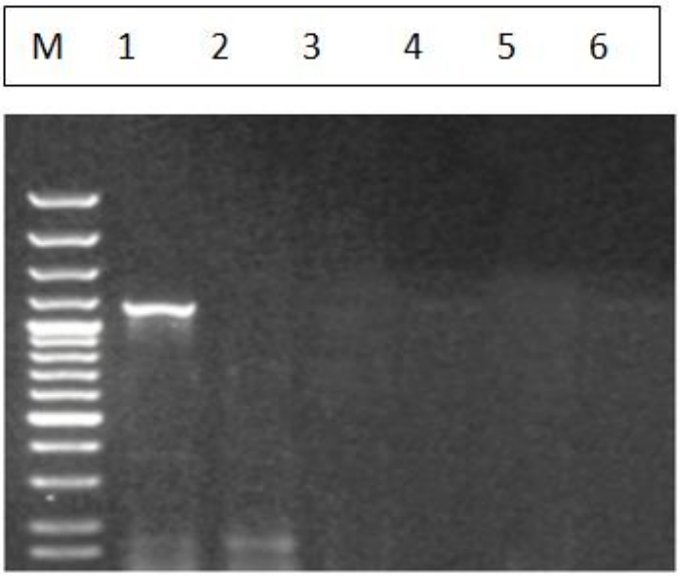


Fig.3 Iodine kit method for detection of citrus greening
a) Healthy leaf sample of sweet orange, b) Citrus greening infected leaf sample, c) sand paper, d) Iodine solution, e) yellow colour development in healthy leaf sample, f) Black colour development in citrus greening infected sample

Fig.4 Detection of citrus greening (HLB) infection in sweet orange (*Citrus sinesis*)



The developed protocol will be helpful for rapid, early, cost effective detection of citrus greening (HLB) infection among citrus species. The development of molecular detection technology resulting in more convenient, effective and specific assays has opened the door to greater use of these tests for detecting plant pathogens.

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Research Article

IN VITRO MICRO PROPAGATION OF SWEET ORANGE

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Abstract: Present investigation was conducted to standardize a protocol for in-vitro propagation of citrus spp. *i.e.* sweet orange for commercial purpose. The shoot tip explant was found better for callus induction of these plants than the nodal segment and epicotyls. Maximum callus formation (40.0% and 23.3% 22.2%) of shoot tip explants was obtained respectively in treatment MS basal media + 0.8mg/l Kinetin, 1.5mg/l NAA, and 2.5 mg/l 2, 4-D. Furthermore, the maximum number of shoots per explant was obtained through the callus in MS basal media + BA 1mg/l. Maximum rooting of shoots (1.11%) was noted sweet orange for the ½ MS media supplemented with 0.2 mg L-1 NAA plus 0.1 mg L-1 BA. Although the callus development and bud proliferation were recorded in all explants however, shoot and root formation did not occur. The potting media composing of soil, sand and FYM in the ratio of 1:1:1 by volume was better with maximum survival rate of hardened plants six weeks after transferring to the pots under greenhouse. In this way we can use this standardized protocol for regeneration of different rootstock for purity and uniformity purpose in seedling.

Keywords: *In-vitro multiplication, Citrus, Sweet orange, Micropopogation*

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Introduction

The citrus has been recognized as one of the most economically important group of plants in the world. Improvement of citrus by conventional method is hampered by polyembryony, sexual incompatibility and male or female sterility [1]. Citrus propagation by conventional means is restricted to particular season and availability of plant material.

It doesn't guarantee trueness of cultivars and mass production of certified Citrus plants throughout the year. Plant tissue culture has emerged as a powerful tool for propagation and improvement of many woody plant species including Citrus. Citrus also stands among difficult to root crops and micropropagation offers rapid propagation of such crops in limited space and time under controlled conditions throughout the year. *In vitro* culture further eliminates diseases [2], provides scope for the development of new cultivars through somaclonal variation [3] and somatic hybridization [4-7], that have improved Citrus rootstock resistance against nematode infestation and other pests as well. Reports on Citrus mircopropagation revealed maximum callus induction percentage in Kinnow (86.8%) on Murashige and Tucker's medium supplemented with 0.01mg/L BA, NAA and 500mg/L malt extract [8]. Different concentrations of growth regulators 10mg/L benzyladenine (BA), 0.1mg/L NAA and 500mg/L malt extract caused maximum initiation of shoot buds from Citrus stem explants grown *in vitro* [9].

The best rooting (100%) in the minimum time (15 days) occurred in the half strength MS medium supplemented with growth hormones (1.0mg/L NAA). The present research work was planned to estimate the effect of growth regulators on the enhancement of growth and development. Further, to induce multiple shoots in Citrus cultivars for mass propagation of certified disease-free plant material. Kinnow has replaced the traditional cultivars of sweet orange due to its outstanding adaptation to agroecology of different region in India, which led to profuse vegetative growth and heavy yield with good fruit quality. Because of these the requirement of disease-free planting material required for plantation purpose.

Source of explant, photoperiodic factors, cut modes, hormonal concentrations and additives may affect *in vitro* citrus shoot regeneration. Epicotyl segments excised from seedlings germinated in the dark for 3 - 6 weeks [10] and then transferred to a 16 h photoperiod that varied from 1 to 3 weeks [11,12] improved the transformation efficiency. For hormones, the effect of auxin on shoot regeneration was rarely concerned, though the main hormone effect on bud formation was due to the addition of BAP [13].

Almeida *et al.* (2003) [14] recorded maximum number of shoots when epicotyl segments were cultured on regeneration EME medium supplemented with 25 g/l additional sucrose and 1 and 2 mg/l BAP for sweet orange and rangpur lime, respectively. Among cut modes, transversal cut, the most popular cut mode [15] is simple to manipulate but produces the fewest adventitious buds. Longitudinal cut, a newly developed but infrequently used cut mode produced the most adventitious buds [16].

Materials and Methods

Plant material and explants preparation

Seeds were extracted from ripe fruits sweet orange. Fruits were collected from a citrus germplasm collection of NRCC, Nagpur. Seed integuments were removed and disinfestation was done with 1% Mercuric Chloride for 5 min. Three washes in distilled and sterilized water were done before the seeds were introduced in culture jars containing 50 ml of MS medium [17], supplemented with 30 g l-1 sucrose. The seeds were maintained at 29 ± 2°C in the dark for three weeks, followed by one, two or three weeks under a 16 h photoperiod.

Explants selected for study

The shoot tip, nodal segment and epicotyls were selected for the study from the sweet orange plant germinated in controlled condition as shown in [Fig-1].

Media and culture conditions

Three different media were used; MS (Murashige and Skoog, 1962) medium supplemented with 500 mg l-1 malt extract and 25 g l-1 sucrose (N1), MS supplemented with 500 mg l-1 malt extract and 40 g l-1 sucrose (N2), and MS supplemented with 500 mg l-1 malt extract, 50 g l-1 sucrose, and 3 mg l-1 BAP (N3). After adjusting the pH to 5.8 ± 0.1 using 1 M NaOH, 1% Difco Bacto agar was added to the media. The media were sterilized by autoclaving at 121°C for 15 min, and 25 ml medium was stored in 100-ml flasks and sealed with Parafilm (American Can, USA). The shoot tip, nodal segment and epicotyls were regularly subcultured on the same fresh medium every 21 days. callus obtained from explants were isolated and cultured in test tubes containing 15 ml solid MS medium with agar (10 g l-1) supplemented with 45 g l-1 sucrose; this medium was with different concentration of hormone. The culture tubes and flasks containing the explants were incubated in a culture room at 25 ± 1°C under 16-h day length with an illumination of 100 µmol m-2 s-1 white 18 W fluorescent lamps. Plantlets were stored for a year under the above-mentioned conditions. The green and healthy callus was transferred on the shooting media in combination with kinetin, NAA, 2, 4-D. After 2-3 weeks regenerated shoots were transferred on the rooting media containing ½ MS with IBA and BAP with different concentration.

A-shoot tip B-Nodal segment C-epicotyl

Fig-1 Explants used in study

A-Seeds of sweet orange B-Callus C-Shooting

D-Multiple shooting E-Rooted shoot F-hardened plant

G-seedlings micropropagated plants

Fig-2 Steps followed in regeneration of Sweet orange

Treatments used

Indirect organogenesis

Two concentrations of BA (2 and 4 mg L-1) with 0.1 mg L-1 NAA were tested in a basal medium for callus induction. In addition, 1 or 2 mg L-1 BA with 0.1 mg L-1 NAA was tested for shoot induction from callus.

Rooting of shoots

The rooting medium contained half strength MS medium supplemented with 0.2 mg L-1 NAA plus 0.1 mg L-1 BA.

Acclimatization

The rooted plantlets were transplanted into 15 cm in diameter pots containing a mixture of sand: peat moss: FYM (1:1:1), placed in a growth room under controlled conditions (temperature 29±2°C,16/8 h photoperiod and light intensity 1500 Lux). The process of acclimatization continued for 8 months, and the rate of survival was 100 % as shown in [Fig-2].

Results

Among the explants selected epicotyls show best results than the other on different concentration of growth media for indirect organogenesis. Under dark/light condition, the buds differentiated from the callus formed at the cut end. Bud formation increased when BA concentration was enhanced. Meanwhile, the number of quiescent shoots regenerated increased. When combined with 0.2 mg/l IbA, the additive effect appeared at 2.0 mg/l of BA. The mean number of buds reached a maximum of 9.8 per explant, among which about five buds could elongate to shoots. Therefore, MBI medium was chosen as the optimal medium for use during micropopogation of sweet orange epicotyls explants

Discussion

In some species, somatic embryos were obtained from special explants cultured on hormone-free media but epicotyls show better regeneration in the medium supplemented with high sucrose and BAP. Regarding root and shoot induction *in vitro* exists in case of Citrus cultivars too. Supplement of both BA (1mg/L) and NAA (10mg/L) in the basal media showed multiple shoot and root formation in sweet orange. BA as a cytokinin was found inhibitory at higher levels for shoot induction in all cultivars leading to the fact that hormone sensitivity was similar for the cultivars studied while NAA when used for root formation did not show any inhibitory response. Such studies might be a promising step towards mass production of sanitized plant material of Citrus.

Application of research: In the present investigation efforts have been made to standardize the protocol for *in vitro* multiplication of sweet orange. It may be possible to use same standardized protocol to propagate some Citrus rootstocks *viz*. Rough lemon, Cleopatra mandarin and other desired rootstocks available *in vitro* if they are desirable enough to justify the labor and expense that would be involved.

Research Category: Micro-propagation

Abbreviations: BAP: Benzyl amino purine, IBA: Indoline butyric acid, MS: Murashige and skoog, NAA: 1-Naphthaleneacetic acid.

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Study area / Sample Collection: NRCC, Nagpur

Cultivar / Variety / Breed name: Citrus

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.
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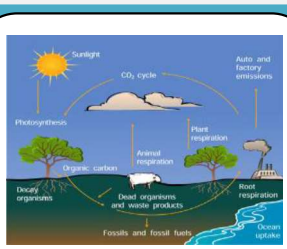

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Application of Nanofertilizer for Sustainable Agriculture Development

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Abstract:

The increasing food demand as a result of the rising global population has prompted the large-scale use of fertilizers, pesticides, herbicides and fungicides etc. As a result of resource constraints and low use efficiency of fertilizers and high use of chemicals, the cost to the farmer is increasing dramatically. Nanotechnology offers great potential of solution to use fertilizer production with the desired chemical composition, improve the nutrient use efficiency that may reduce environmental impact, and boost the plant productivity with soil quality. Furthermore, controlled release and targeted delivery of nanoscale active ingredients can realize the potential of sustainable and precision agriculture. The present article concludes that the application of nanofertilizers for the sustainable development of agriculture. It is largely contributing to the growth and development of agricultural and horticultural crop in future. It provides the sustainability to agriculture.

Keywords: *Nano, Nanofertilizer, Sustainability, Agriculture, Nanoparticles etc*

Introduction:

Fertilizers have been used for the past many years in agriculture for the benefit of farmers to have high yield. Traditional fertilizers are expensive as well as harmful to human beings and the environment. Therefore, there is a need for developing environment-friendly fertilizers having high nutrient value as well as compatibility with soil and environment. Nanotechnology plays a significant role in promoting agriculture and agricultural products. Agriculture and food industry aims for the sustainability and the protection of agricultural products, including crops for human and livestock. It helps in the manufacturing of innovative agrochemicals and novel delivery mechanisms to enhance crop production and decrease the use of chemical fertilizers and pesticides. It acts as an important tool in agriculture to improve crop growth, yield, and quality parameters with increased nutrient use efficiency, reduced wastage of fertilizers and cost of cultivation(1-3).

Nanotechnology is a field of research and innovation concerned with building 'things' - generally, materials and devices - on the scale of atoms and molecules. A nanometre is one-billionth of a metre: ten times the diameter of a hydrogen atom. The diameter of a human hair is, on average, 80,000 nanometres (4). There are lots of applications of nanotechnology as shown in fig.1.

The rapid growth in the world population has increased the demand from the agricultural sector, making researchers wary of the overuse of chemical fertilizers by farmers. Nanofertilizers have emerged as a promising alternative that ensures high crop production and soil restoration. **Nanofertilizers** are being prepared by encapsulating plant nutrients into nanomaterials, employing thin coating of nanomaterials on plant nutrients, and delivering in the form of nano-sized emulsions (5-7).

These nanostructures have shown slow degradation and controlled release of active ingredient for long time. Because of the limitation in arable lands and water resources, the development of agriculture sector is only possible by increasing resources use efficiency with minimum damage to production bed through effective use of modern technologies (8). These nano-agro-formulations increase nutrient use efficiency, reduce soil toxicity, minimize the potential negative effects associated with over dosage, and reduce the frequency of the application (9).

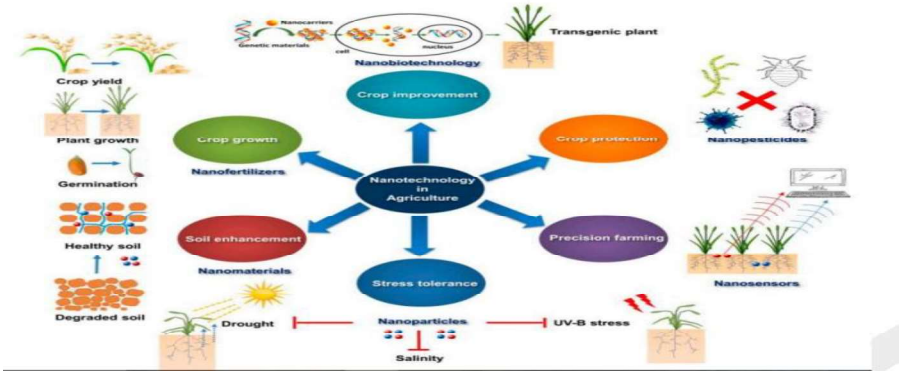

Fig. 1 Applications of nanotechnology in agriculture (24)

The growing population and a narrowing cultivatable land base and water resources create the demand in agriculture for greater efficiency in food production. Natural or synthetic fertilizers are utilized in the soil–crop systems to fulfil the essential macronutrients and micronutrients nutrient requirement of the plants and boosting crop yield [10]. Farmers applied commercial fertilizers to crop plants for the last 50 years for optimum plant growth that maintain the balanced distribution of the three primary macronutrients such as nitrogen (N), phosphorous (P), and potassium (K) and three secondary macronutrients like Sulfur (S), magnesium (Mg), and calcium (Ca). However, micronutrients like selenium (Se), boron (B), molybdenum (Mo), manganese (Mn), chlorine (Cl), copper (Cu), iron (Fe), and Zinc (Zn) are needed in low amounts for plant growth. The primary macronutrients are required to apply externally due to their inadequate nature in soil [2]. The most used commercial fertilizers are urea, triple superphosphate (TSP), diammonium phosphate (DAP), single superphosphate (SSP), monoammonium phosphate (MAP), and nitrogen–phosphorous–potassium (NPK), which contain essential plant nutrients such as nitrogen, potassium, and phosphorus [11]. Nitrogen is the first and fore most required nutrient for crop plants among mineral nutrients that is the integrant of many enzymes and proteins and chlorophyll thus critical to vegetative growth of crops. The utilization efficiency of nitrogenous fertilizers is only 45–50%, and for phosphorous fertilizers is only 10–25% [12]. It has been reported as in early 1970, only 27 kg NPK ha−1 was needed for one ton of grain production, whereas in 2008 increased to 109 kg of NPK ha−1 to gain the same amount of production. The International Fertilizer Industry Association (IFIA) reported that world consumption of fertilizer has been rising sharply, and in the year 2016–2017, demand was projected to 192.8 Mt [13]. That runoff overdosing of chemical fertilizers leads to eutrophication in aquatic ecosystems, i.e. the growth of algal on the water surface due to the enriched nutrients on water, which make a barrier to oxygen supply to living organisms into water. Commonly, the utilization efficacy of mineral fertilizers or applied chemicals has remained below 30% [14]

Inefficient fertilizer management leads to environmental pollution, climate change, and economic consequences. For example, approximately half of the applied nitrogen fertilizer lost from agricultural fields to air, water, and other processes that lead to a negative impact on the environment like N-oxides release into the atmosphere thus being as greenhouse gases and lead global warming, and nitrates leached into marine ecosystems [15].

Why is the Use of NanoFertilizers Better than Conventional Fertilizers

The unique properties of nanoparticles, such as high sorption capacity, the increased surface to volume ratio, and controlledrelease kinetics to targeted sites, make them a potential plant growth enhancer. Because of these characteristic features, nanostructured fertilizers can be used as a smart delivery system of nutrients to the plant. Nanofertilizers are released very slowly in comparison to conventional fertilizers (16). This approach improves nutritional management, i.e., increasing the nutrient use efficiency and decreasing nutrient leaching into groundwater. Nanofertilizers are specifically designed to release active ingredients in response to biological demands and environmental stress. Scientists have further stated that nanofertilizers increase agricultural productivity by improving photosynthetic activity, seedling growth, rate of seed germination, nitrogen metabolism, and carbohydrate and protein synthesis (17).

Nanofertilizers:

For plant nutrition, sufficient amount of macro- and micronutrients is necessary, including carbon, oxygen, hydrogen, nitrogen, phosphorus, potassium, calcium, sulfur, and magnesium. Out of these, the first three are structural elements and extracted from the environment, while the remaining six are extracted from soil. Though all the macronutrients are important, yet primary macronutrients are consumed in higher quantities in comparison to secondary ones (18). These primary macronutrients (nitrogen, phosphorus, potassium) are considered fertilizer elements as the familiar "N-P-K" identified on fertilizer labels.

Advantages Of Nanofertilizers Over Conventional Mineral Fertilizers

Nanofertilizers offer lots of benefits for sustainable and eco-friendly crop production more. Some of the advantages are (4, 19);

1. Nanofertilizers lead to the absorption and utilization of efficient nutrients without higher losses.
2. Nanofertilizers reduce the risk of environmental pollution via reduce the losses of nutrients.
3. Comparatively nanofertilizers have higher diffusion and solubility than conventional synthetic fertilizers.
4. Nanofertilizers deliver nutrients gradually to crop plants in a controlled manner which is in total conflict with the spontaneous and rapid delivery of nutrients from chemical fertilizers.
5. Nanoparticles can be easily uptake into plants via nano-sized porous, and by molecular transporters as well as root exudates. Nanoparticles uptake higher nutrient by plants via using various ion channels.

6. Smaller amounts of nanofertilizers are enough to apply than synthetic fertilizers due to their small loss nutrient nature.
7. Polymer-coated fertilizers prevent premature contact with water and soil, and negligible loss of nutrients.
8. Nanofertilizers improve soil fertility and develop a feasible environment for microorganisms (20).

Mode of action of nanofertilizers in field:

Many facets of plant biology structures like the nutrient gateway to the plant and plant roots are on a nanometer scale. Plant cell walls have 5 to 20 nm diameters range pore. one to a few tens of nanometers pores in diameter have been detected in roots for ionic and molecular transport processes. However, nanofertilizers could uptake through these nano-scale pores, or uptake by complexation with root exudates or molecular transporters via new pores creation, or by the exploitation of ion channels endocytosis (21).

Researchers have stated that the plant root system, which is the gateway for the nutrients, is highly porous to nanomaterials (nanofertilizers) than conventional fertilizers. Stomatal openings in leaves are also reported to favor uptake of nanomaterials and their entry to leaves. Scientists have conducted experiments using the faba bean (*Vicia faba*), to determine the nanoparticle's efficiency to penetrate the plant system. They found that nanoparticles (43 nm in size) could penetrate leaf in large numbers compared to nanoparticles larger than 1.0 nm size. Nanofertilizers are also reported to deliver nutrients through plasmodesmata. Plasmodesmata are nanosized channels of approximately 50–60 nm size used to transport ions between cells. Carbon nanotubes and silica nanoparticles are useful tools for transporting and delivering cargoes (nutrients and other important biochemicals) to plants' target site (22). The uptake of nanofertilizer by plant is shown as in fig.2.

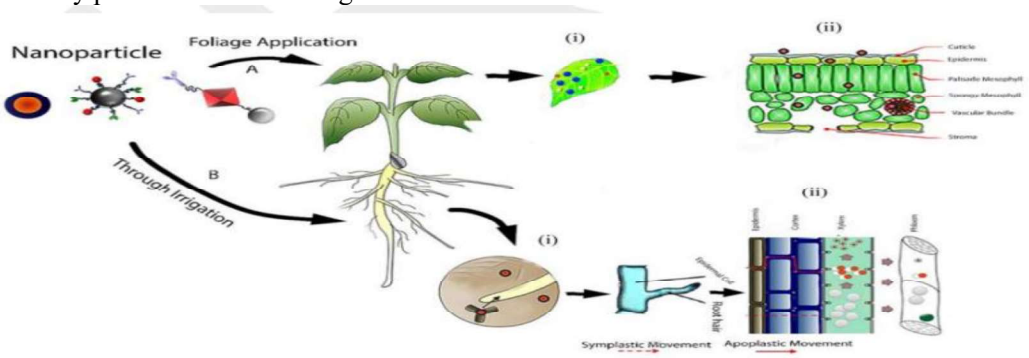

Fig.2 Uptake and translocation mechanisms of nanoparticles in a plant through leaf and roots (10)

Use of NanoFertilizers in Sustainable Crop Development

Group of scientists believe that zinc nanofertilizers are responsible for robust plant growth (shoot and root system) and increase the leaves' chlorophyll content. In a previous study, the amendment of zinc nanofertilizers significantly increased the yield of peanuts. These nanofertilizers also improve seed production of vegetables. Similarly, carbon nanotubes containing fertilizers were reported to decrease the days to germination. These nanofertilizers were also found to promote the development of plant root systems in rice seedlings. Nanofertilizers also reduce the crop cycle period and increase crop yield. For example, the amendment of nanoparticles carrying NPK (nitrogen, phosphorus, and potassium) to wheat showed an increase in grain yield and reduced the crop cycle of wheat by 40 days. Application of nanobiofertilizers has been studied to significantly improve the crop growth through the optimization of photosynthesis, nutrient absorption efficacy, higher photosynthate accumulation and nutrient translocation, enabling enhanced productivity as well as quality. One study has specifically reported the characteristic effect of nanobiofertilizers made via entrapment of biofertilizer (growth promoting bacteria) within Au and Ag NPs, wherein significantly higher crop growth was witnessed upon the administration of NPs with bacteria compared to those with the NPs alone. Nano structured fertilizer consisting of neem cake with PGPR provides efficacy toward promoting crop-harvest yields in several leguminous crops through an earlier and greater seed germination as well as effective delivery of doped nutrients (9,23).

Conclusion

Nanofertilizers are emerging as a promising alternative to chemical fertilizers in agriculture. Nanofertilizers may exert a positive role through slow nutrient releasing and it helps plant for increased in nutrient use efficiency. Nanofertilizers help to increase in tolerance to abiotic stress in several cases

Nanofertilizers are utilized alone or in conjunction with organic materials to efficiently boosting nutrients to crop plants while reducing environmental pollution via minimize nutrient loss and enhance the

higher absorption rate. Several types of research with different nanomaterials were recorded to enhance the root development, plant height, germination rate, number of roots, and fruits antioxidant and leaf chlorophyll contents. Smart nanofertilizers release nutrients as per the requirements of plants for sustainable crop production. Lastly, researchers and regulators should be responsible for the risk and limitation of nanofertilizer usage in order to take full advantage of nanofertilizers for sustainable crop production under changing climate while reducing the risk of causing environmental pollution.

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