RESEARCH ARTICLE Legume Research- An International Journal, Volume 47 Issue 1: 14-19 (January 2024)

Influence of Nano Formulation on Augmenting the Seed Quality in Groundnut (*Arachis hypogaea* L.)

C. Tamilarasan[1], K. Raja[1]

10.18805/LR-4487

ABSTRACT

Background: Groundnut were mainly cultivated under rain fed condition, lack of initial seedling quality due to lower moisture conditions, it causes reduced crop yield and in sometimes it leads to crop failure. In order to overcome this problems, effective seed enhancement techniques as seed coating plays major role in maintaining the seed quality.

Methods: The controlled release of bioactive molecules namely hormones gibberellic acid (GA_3) as a smart delivery system can be achieved through seed coating in groundnut by exploiting advanced nanotechnology. Infusion of active ingredients as hormones (GA_3) through the seed coating formulation by nano formulation methods. At nano level it influences the effects at higher level and gives best results through controlled release of hormones without any loss.

Result: Due to nano formulation coating in groundnut, seeds had an effective results in all the parameters *viz*., imbibition rate (48%), speed of germination (5.5), germination percentage (87%), seedling length (24.7 cm), vigour index (2142), hydrolysing enzymes (α-amylase 14.83 and lipase 1.481), which significantly differs from untreated seeds. Hence, it was concluded that the GA_3 infused nano formulation coating at 15 ml per kilogram of seed can be used for seed quality improvement in groundnut for better seedling establishment.

Key words: Gibberellic acid, Groundnut, Hormones, Nano formulation, Seed quality.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the most important oil seed crop often referred as the “king of oil seed crops”. In India, nearly 70 per cent of groundnut is cultivated as rainfed crop where assured germination and vigorous plant establishment decide the crop yield. In the world 90 percentage of groundnut is being cultivated using quality seeds as it accounts an important role in crop production. Plant growth hormone (GA_3) promote germination, seedling vigour and plant growth, thus maximizing yield and quality. Gibberellic acid (GA_3) induces the synthesis of α-amylase and hydrolysis of starch resulting in germination of seeds (Palmiano and Juliano, 1972).

Gibberellins are capable of inducing a range of genes, which are necessary for the production of hydrolytic enzymes that include α-amylase, protease, lipase and α-glucanase which act on the stored food reservoir resulting in energy production and supply to the growing embryo during germination (Yamaguchi, 2008). Tamilarasan *et al*. (2018) reported that application of IAA loaded nano formulation on seed quality invigorated the physiological and biochemical qualities. However, these potential benefits of hormones are not fully expressed in plant system due to the biological shortcomings of very minimum quantity of hormones application. One of the novel approaches to develop new and more effective hormones is modification of compounds to attain controlled release. With respect to environment, the use of controlled release of hormones often reduces losses and improves the efficiency.

Moreover, groundnut is bold seeded and coating technology is less popular due to fact that the testa is very delicate and prone to pre-disposed damages. To avoid damages and loss of viable seeds by coating, in groundnut seed coating technology is restricted with bio-inoculants and dry dressing with fungicides and nothing have been done to infuse other inputs through pelleting and seed coating. In order to deliver inputs for seed invigouration in groundnut the new approaches as nano-formulation coating was introduced which also plays role in maintaining the optimal release of inputs to germinating seeds. The controlled release of bioactive molecules namely hormones as a smart delivery system can be achieved through seed coating in groundnut by exploiting advanced nanotechnology. Jyothi and Hebsur (2017) reported that application of nano fertilizers may help in slow and steady release of nutrients and it also minimize the loss of nutrients and improves nutrient use efficiency. Also, Raj *et al*. (2021) reported that application of Zinc sulphate nano particle as seed priming invigoration improved the seedling growth and establishment. The right seed coating plays a major role, wherein every coated seed has the opportunity to fulfil its

[1]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: C. Tamilarasan, Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
Email: tamilarasan2510@gmail.com

How to cite this article: Tamilarasan, C. and Raja, K. (2024). Influence of Nano Formulation on Augmenting the Seed Quality in Groundnut (*Arachis hypogaea* L.). Legume Research. 47(1): 14-19. doi: 10.18805/LR-4487.

Submitted: 20-08-2020 **Accepted:** 05-11-2020 **Online:** 29-12-2020

potential, which could be achieved through encapsulation of seeds with bioactive inputs incorporated nanoformulation. To overcome the coating damages in groundnut and also to provide exact growth elements to emerging seeds the following study was formulated. Therefore, the main objective of the study was to develop coating formulation and to fix the optimum doses of nano-formulation for effective germination and seedling establishment.

MATERIALS AND METHODS

Experiment was carried out during 2017 at Department of Nano Science and Technology and Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Freshly harvested and genetically pure seeds of groundnut TMV 13 were obtained from Oilseeds Research Station, Tindivanam. The seeds were dried, cleaned, size graded using round perforated sieve (24/64) and tested for initial seed quality under laboratory conditions. Hormones (gibberellic acid), polymer (Carboxymethyl cellulose) and surfactant (Tween 80) were purchased from M/s. Sigma Aldrich Chemicals Private Limited, Bangalore, India. These seeds, hormones and chemicals constituted as study materials for the present investigation.

Conventional emulsions of CMC based GA_3 loaded, using surfactant Tween 80 were prepared by adopting organic solvent displacement technique (Solans *et al*., 2005). To prepare 100 ml of 100 ppm GA_3 loaded formulation, CMC, GA_3 and Tween 80 were mixed at the ratio of 79: 20: 1. Conventional emulsion CMC based GA_3 loaded (CMC 1% + GA_3 100 ppm + Tween 80 1%), prepared by organic solvent displacement method were subjected to high energy homogenization where the pressure ranged from 10000 bar to 40000 bar with an interval of 10000 bar (Sharma *et al*., 2014). Then, the homogenized nano formulation were used for seed coating at four different doses as T_0- Control, T_1- 5 ml of nanoformulation kg^{-1}, T_2 - 10 ml nanoformulation kg^{-1}, T_3- 15 ml nanoformulation kg^{-1}, T_4- 20 ml nanoformulation kg^{-1} and seeds was evaluated for physiological and biochemical parameters with five replication in each treatments.

The following observations were observed:

Imbibition rate

Twenty-five coated seeds from each replication were taken and placed in petri plates inner layered (two layer) with moist germination paper and the Petri dishes were kept at room temperature for 24 h. Increase in seed weight was measured every two hours. The imbibed seeds were removed and wiped out with tissue paper to remove the adhering water droplets and were weighed for their final weight on a top pan balance. Based on the difference between the weights, the imbibition rate was calculated and the mean was expressed in percentage. The data observed were analysed in factorial completely randomized block design (Sundaralingam, 2005).

Speed of germination (Maguire, 1962)

The germinated seedlings from each replications of treatments were counted daily from 1st day onwards up to 10th day of sowing. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula and the result was expressed in number:

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots \frac{X_n - X_{n-1}}{Y_n}$$

Where,

X_1- Number of seeds germinated at first count.
X_2- Number of seeds germinated at second count.
X_n- Per cent germination on n^{th} day.
Y_1- Number of days from sowing to first count.
Y_2- Number of days from sowing to second count.
Y_n- Number of days from sowing to n^{th} count.

Germination (%)

The germination per cent was observed from the site of experiment in four replications to test 400 seeds as per (ISTA, 2015) rules. The germination rate measures the time course of the seed germination. At the end of the 25th day the final count was taken and the number of normal seedlings were recorded.

$$\text{Germination per cent} = \frac{\text{Seed balls germinated}}{\text{Total seed balls}} \times 100$$

Root length (cm)

At the time of final count, ten normal seedlings were randomly selected from each replication and root length of each seedling was measured. The measure from the point of attachment of the seed to the tip of the primary root was taken as root length.

Shoot length (cm)

The same seedlings used to measure root length were used for measuring the shoot length and was measured from the point of attachment to the tip of the leaf.

Dry matter production (g per seedlings^{-10})

The seedlings selected for the root and shoot length were placed in a paper cover, shade dried for 24 h and were dried at 80°C for 16 ±1 h in a hot air oven. Then they were cooled in a desiccator, weighed and expressed as mg 10 $seedlings^{-1}$.

Vigour index

Vigour index were computed using the following formula and the mean values were expressed as a whole number (Abdulbaki and Anderson, 1973):

Vigour index = Germination (%) × Total seedling length (cm)

α-amylase activity (mg maltose min^{-1})

The seeds from each of the treatments and replications were germinated adopting top of the paper method upto radical protrusion. From the pre-germinated seeds 500 mg was weighed and homogenized in 1.8 ml of cold 0.02 M sodium phosphate buffer (pH 6.0) and centrifuged at 20,000 rpm for 20 min. to extract the enzymes. To the 0.1 ml of enzyme extract, one ml 0.067per cent starch solution was added. The reaction was stopped after 10 min. of incubation at 25°C by addition of one ml of iodine HCl solution (60 mg KI and 6 mg

I2 dissolved in100 ml of 0.05 NHCl). The colour change was measured at 620 nm using a double beam spectrophotometer 2205. The amylase activity was calculated by adopting the following formula and the mean values were expressed in mg maltose min^{-1} (Paul *et al*., 1970):

$$\alpha\text{-amylase activity} = \frac{\text{OD value}}{\text{Volume of sample pipetted out}} \times \frac{1000}{500}$$

Lipase activity (meq min^{-1} g of $sample^{-1}$)

A. Preparation of enzyme extract

Two grams of pre germinated seeds from each replications of treatments was taken, ground and homogenized with twice the volume of ice-cold acetone. Then powder was washed successively with acetone, acetone:ether (1:1) and ether, dried and stored in refrigerator till used. Extract was obtained by centrifuging 1 g of powder in 20 ml of ice-cold water at 15, 000 rpm for 10 min.

B. Substrate

Two milli litre of clear vegetable oil (pH 7.0), 25 ml of water and 100 mg of bile salt (Sodium taurocholate) was added and stirred well till a formulation was formed. Further, two gram of gum Arabic was added to hasten emulsification.

Five milli litre phosphate buffer (pH 7.0) was added to 20 ml of substrate taken in a 500 ml beaker and the contents were stirred slowly using magnetic stirrer cum hot plate maintained at a temperature of 35°C and then the pH was adjusted to 7.0. To the mixture, 0.5 ml of enzyme extract was added and the pH was recorded immediately and the timer was set on. When pH dropped by 0.2 unit, 0.1 N NaOH was added to bring the pH to the initial value and the titration was continued for 30 min. The enzyme activity was calculated from the amount of alkali consumed using the following formula and expressed in meq min^{-1}g of $sample^{-1}$ (Sadasivam and Manickam, 2008):

$$\text{Lipase activity} = \frac{\text{Vol. of alkali consumed}}{\text{Weight of sample in g}} \times \frac{\text{Strength of alkali}}{\text{Time in min}}$$

RESULTS AND DISCUSSION

The results obtained from all treatments had an effective difference in all the parameters due to hydrophilic polymer nano formulation coating.

Imbibition rate

Imbibition rate was significantly influenced by CMC based GA_3 loaded nano formulation coating with different concentrations over a period of 12 h of imbibition. The maximum imbibition rate of 74.40% was observed in seeds coated with 15 ml kg^{-1} at 12 h which had no significant difference with 10 ml kg^{-1} (73.80 per cent). The minimum was recorded in control seeds (67.00%) at 12 h of imbibition (Fig 1). Higher rate of imbibition rate was due to nano formulation coating, polymer having a capability of water absorption even under very low moisture content. It induces the fast activation of hydrolysing enzymes which are involved in seed germination process. Fastest absorption of water also hastens the speed of germination and better seedling establishments, which was supported with the following research findings (Koornneef *et al*., 2002; Subash *et al*., 2014 and Sakthivel, 2016).

Speed of germination

Significant difference was observed in speed of emergence due to CMC based GA_3 loaded nano formulation coating, due to fastest absorption of water, it influences the germination speed. The highest value of 5.5 was observed in 15 ml kg^{-1} while the control recorded the lowest value (3.2) (Fig 1, Table 1). Metabolic process repair and build-up of metabolites occurred during seed in vigouration may be the plausible reason for higher germination (Chen and Arora, 2013; Jisha *et al*., 2013).

Germination (%)

Germination per cent was significantly influenced by different concentrations of GA_3 loaded nano formulation. Among the different concentrations, 15 ml kg^{-1} of seed (T_3) recorded the maximum germination 87 per cent which was on par with 10 ml kg^{-1} of seed (T_2), which recorded 85 per cent of germination. The untreated control (T_0) registered the lowest germination 76 per cent (Table 1). The improvement in

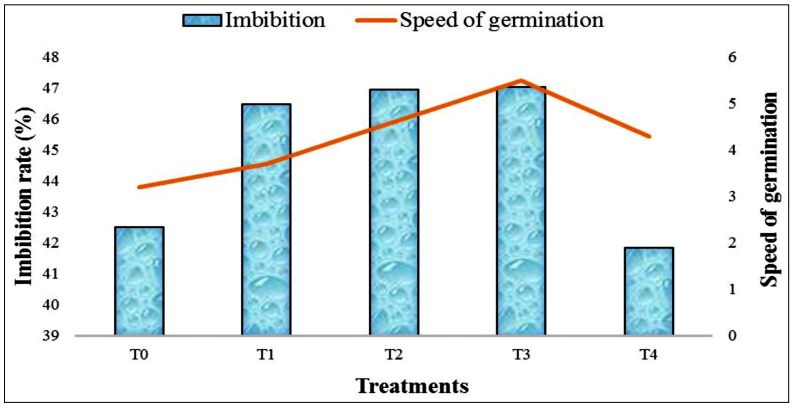


Fig 1: Influence of GA_3 loaded nano formulation seed coating on imbibition rate and speed of germination.

Table 1: Physiological changes in groundnut seed coated with nano formulation.

Treatments	Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index	DMP (gm / 10 seedlings)
T_0	3.2	76 (60.66)	7.9	11.8	1497	2.748
T_1	3.7	80 (63.43)	8.4	14.2	1808	2.768
T_2	4.6	85 (67.21)	8.9	14.7	2006	3.177
T_3	5.5	87 (68.57)	9.4	15.3	2142	3.451
T_4	4.3	82 (64.89)	8.5	13.6	1812	3.098
Mean	4.3	82 (64.89)	8.6	13.9	1853	3.048
SEd	0.065	1.063	0.138	0.118	25.803	0.033
CD (P=0.05)	0.139	2.125	0.295	0.251	54.100	0.071

(Figure in parenthesis indicate arcsine values). T_0- Control, T_1- 5 ml of nano formulation kg^{-1}, T_2- 10 ml nano formulation kg^{-1}, T_3- 15 ml nano formulation kg^{-1}, T_4- 20 ml nano formulation kg^{-1}.

Table 2: Biochemical changes in groundnut seed coated with nano formulation.

Treatments	α-amylase activity (mg maltose min^{-1})	Lipase activity (meq min^{-1} g of $sample^{-1}$)
T_0	11.82	0.895
T_1	13.08	1.190
T_2	14.26	1.416
T_3	14.83	1.481
T_4	13.48	1.186
Mean	13.494	1.234
SEd	0.132	0.019
CD (P=0.05)	0.281	0.040

T_0- Control, T_1- 5 ml of nano formulation kg^{-1}, T_2- 10 ml nano formulation kg^{-1}, T_3- 15 ml nano formulation kg^{-1}, T_4- 20 ml nano formulation kg^{-1}.

germination of CMC coated seeds may be attributed to the activation of cells and enhancement of mitochondrial activity which is closely associated with the formation of energy compounds and vital biomolecules that are required during the early phase of germination (Clayton, 1988; Ghiyasi *et al*., 2008 and Jisha *et al*., 2013). Devishri (2012) who has reported that carboxylmethyl cellulose coating had improved germination in cotton by increasing the imbibition and water for a longer period of time.

Root length (cm)

Root length of germinated seeds was significantly differed due to nanoformulation coating. Seeds coated with nano formulation at 15 ml kg^{-1} of seed (T_3) had produced the longest root length (9.4 cm) followed by 10 ml kg^{-1} of seed coating, which had produced the root length of 8.9 cm whereas control has produced root length of having 7.9 cm (Table 1). Naruka *et al*. (2000) and Amanullah *et al*. (2008) also observed that same result as increased root length and secondary roots.

Shoot length (cm)

Shoot length of germinated seeds was significantly differed due to nanoformulation coating. Among the different concentrations, seeds coated with nanoformulation at 15 ml kg^{-1} of seed (T_3) have produced the longest shoot length (15.3 cm) followed by 10 ml kg^{-1}, which has produced the shoot length of 14.7 cm whereas untreated seeds showed shoot length of 11.8 cm. (Table 1). The result was on par with the following findings application of nano particles at low concentrations improves the cell division and elongation in shoot growth (Ali *et al*., 2019 and Asl *et al*., 2019).

Vigour index

The results of vigour index showed significant difference due to CMC based GA_3 loaded nanoformulation seed coating. Formulation at the rate of 15 ml kg^{-1} of seed (T_3) had recorded the maximum vigour index of 2142, which was followed by 10 ml kg^{-1} of seeds (2006). Untreated seeds have recorded the minimum (1497) vigour index (Table 1). Devishri (2012) who has reported that carboxylmethyl cellulose coating had improved seedling vigour in cotton by retaining water for a longer period of time.

Dry matter production (g per seedlings^{-10})

Dry matter production was significantly differed in respect to the different concentration of nanoformulations coating. The seeds coated with CMC based GA_3 loaded nanoformulation at 15 ml kg^{-1} of seed recorded the highest dry matter content of 3.451 g per 10 seedlings. Seeds coated with formulation at 10 ml kg^{-1} of seed had registered the dry matter production of 3.177 g per 10 seedlings. The control seeds recorded the lowest dry matter production of 2.748 g per 10 seedlings (Table 1). The above result of dry matter accumulation was supported with the following findings (Valenciano *et al*., 2010; Usman *et al*., 2014).

α-amylase activity

Seed coating with nano formulation had significant impact on α-amylase activity. Maximum amount of α-amylase activity was measured in seeds coated with CMC based GA_3 loaded nanoformulation at the rate 15 ml kg^{-1} (T_3) (14.83 mg maltose min^{-1}) followed by 10 ml kg^{-1} (T_2) (14.26 mg maltose min^{-1}). Minimum value of (11.82 mg maltose min^{-1}) was registered in uncoated seeds (Table 2). GA_3 triggered the hydrolytic enzymes which helps in early seed germination and growth (Damaries *et al*., 2019).

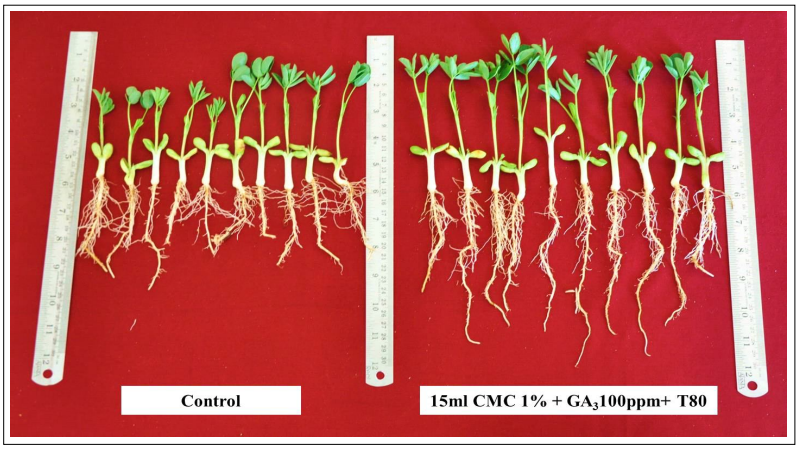


Fig 2: Influence of nano formulation coating on seedling growth.

Lipase activity

Lipase activity was influenced by different concentrations of GA_3 loaded formulation. Among the different concentrations, seeds coated with CMC based GA_3 loaded nanoformulation at the rate 15 ml kg^{-1} (T_3) was found to be recorded the maximum lipase activity of 1.481 meq $min^{-1}g$ of $sample^{-1}$, which had no significant difference with 10 ml kg^{-1} (T_2) (1.416 meq $min^{-1}g$ of $sample^{-1}$). Untreated control had the lowest lipase activity of 0.895 meq $min^{-1}g$ of $sample^{-1}$ (Table 2). Ido and Susno (1990) and Appleford and Lenton (1997) reported the same results of increased hydrolytic enzymes in seeds by nano formulation coating.

Moreover, the hydrolytic enzymes α-amylase and lipase activities were more in seeds coated with 15 ml kg^{-1} of seeds that helped in increased germination and seedling vigour. In addition to that, the increased rate of germination, seedling vigour and hydrolytic enzymes activity was due to faster uptake of water through carboxymethyl cellulose coated seeds by hydrophilic nature of coated material (Fig 2).

CONCLUSION

It is concluded that seeds coated with CMC based GA_3 loaded nano formulation, at the rate 15 ml kg^{-1} of seed recorded higher rate of imbibition (48%), speed of emergence (5.5), germination (87%), seedling length (24.7 cm), vigour index (2142) and dry matter production (3.451) with optimum coating efficiency. It was followed by 10 ml kg^{-1}. Hence, these two concentrations were screened and it can be used for improving seed quality in groundnut for getting better seedling quality.

ACKNOWLEDGEMENT

We acknowledge our grateful thanks to the Department of Nano Science and Technology, TNAU, Coimbatore for financial support for analytical works.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Abdul-Baki, A.A. and Anderson, J.D. (1973). Vigour determination of soybean seeds by multiple criteria. Crop Science. 13: 630-633.

Ali, S., Rizwan, M., Noureen, S., Anwar, S., Ali B., Naveed, M., Abd Allah, E.F., Alqarawi, A.A. and Ahmad, P. (2019). Combined use of biochar and zinc oxide nanoparticle foliar spray improved the plant growth and decreased the cadmium accumulation in rice (*Oryza sativa* L.) plant. Environ. Sci. Pollut. Res. 26: 11288-11299. doi: 10.1007/s11356-019-04554-y.

Amanullah, H., Rahman, Z. and Shah, P. (2008). Plant density and nitrogen effects on growth dynamics and light interception of maize. Arch. Agro. Soil Sci. 54: 401-411.

Appleford, N.E.J. and Lenton, J.R. (1997). Hormonal regulation of α-amylase gene expression in germinating wheat (*Triticum aestivum*) grains. Physics Plantarum. 100(3): 534-542.

Asl, K.R., Hosseini, B., Sharafi, A., Palazon, J. (2019). Influence of nano-zinc oxide on tropane alkaloid production, h6h gene transcription and antioxidant enzyme activity in *Hyoscyamus reticulatus* L. hairy roots. Eng. Life Sci. 19: 73-89. doi: 10.1002/elsc.201800087.

Chen, K. and Arora, R. (2013). Priming memory invokes seed stress tolerance. Environmental and Experimental Botany. 94: 33-45.

Clayton, P.B. (1988). Seed treatment technology - The challenge ahead for the agricultural chemicals industry. British Crop Protection Council Monograph. 39: 247-256.

Damaris, R.N., Zhongyuan, L., Yang, P. and Dongli, H. (2019). The rice alpha-amylase, conserved regulator of seed maturation and germination. International Journal of Molecular Science. 20: 450; doi: 10.3390/ijms20020450.

Devishri, D. (2012). Development of seed tape technology in tomato (*Lycopersicum esculentum*) and onion (*Allium cepa*). M.Sc., (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Ghiyasi, M., Seyahjani, A.A., Tajbakhsh, M., Amirnia, R. and Salehzadeh, H. (2008). Effect of osmopriming with polyethylene glycol (8000) on germination and seedling growth of wheat (*Triticum aestivum* L.) seeds under salt stress. Res. J. Biol. Sci. 3(10): 1249-1251.

Ido, Y. and Susono (1990). Germination promoter and sowing method for seeds of plant. United Patent Office. Patent no: US 5,988,082.

ISTA, (2013). International Rules for Seed Testing. The International Seed Testing Association, Basssersdorf, Switzerland.

Jisha, K.C., Vijayakumari, K. and Puthur, J.T. (2013). Seed priming for abiotic stress tolerance: An overview. Acta Physiology of Plant. 35(5): 1381-1396.

Jyothi, T.V. and Hebsur, N.S. (2017). Effect of nanofertilizers on growth and yield of selected cereals - A review. Agricultural Reviews. 38(2): 112-120. doi: 10.18805/ag.v38i02.7942.

Koornneef, M., Bentsink, L. and Hilhorst, H. (2002). Seed dormancy and germination. Current Opinion in Plant Biology. 5(1): 33-36.

Maguire, J.D. (1962). Speed of germination in selection evaluating for seedling emergence and vigour. Crop Science. 2: 176-177.

Naruka, I.S., Gujar, K.D. and Lal, G. (2000). Effect of foliar application of zinc and molybedenum on growth and yield of okra [*Ablemoschus esculentus* (L.) Moench cv. pusa sawani. Haryana]. Hort. Sci. 29(3 and 4): 266-267.

Palmiano, E.P. and Juliano, B.O. (1972). Biochemical changes in the rice grain during germination. Plant Physiology. 49(5): 751-756.

Paul, A.K., Mukh Erji, S. and Sircar, S.M. (1970). Metabolic changes in rice seeds during storage. Indian Journal of Agricultural Science. 40(12): 1031-1036.

Raj, A.B., Raj, S.K., Prathapan, K., Radhakrishnan, N.V. and Swadija, O.K. (2021). Effect of seed invigouration on yield enhancement in grain cowpea [*Vigna unguiculata* (L.) Walp]. Legume Research. 44(9): 1118-1123. doi: 10.18805/LR-4213.

Sadasivam, S. and Manickam, A. (2008). Estimation of Lipase Content. Biochemical Methods. New Age International Ltd., New Delhi. 102-103.

Sakthivel, C.P. (2016). Nano-encapsulation of active ingredients to improve seed quality of cotton. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.

Sharma, A., Karn, R.K. and Pandiyan, S.K. (2014). Synthesis of TiO_2 nanoparticles by Sol-gel method and their characterization. Journal of Basic and Applied Engineering Research. 1: 1-5.

Solans, C., Izquierdo, P., Nolla, J., Azemar, N. and Garcia-Celma, M.J. (2005). Nanoemulsions. Current Opinion Coll Interface Science. 10: 102-110.

Subash, M., Haseena, R. and Lalitha, J. (2014). Influence of GA_3 and IAA and their frequency of application on seed germination and seedling quality characters. International Letters of Natural Science. 30: 44-48.

Sundaralingam, K. (2005). Organic Seed Production in Hybrid Rice ADTRH 1. Ph.D. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.

Tamilarasan, C., Raja, K., Subramanian, K.S. and Selvaraju, P. (2018). Synthesis and development of nano formulation for hastening seed quality in groundnut. Res. Jr. of Agril. Sci. 10(1): 50-57.

Usman, M., Tahir, M. and Majeed, M.A. (2014). Effect of zinc sulphate as soil application and seed treatment on green gram (*Vigna radiata* L.). Pakistan Journal of Life Social Science. 12(2): 87-91.

Valenciano, J.B., Boto, A. and Marcelo, V. (2010). Response of chickpea (*Cicer arietinum* L.) yield to zinc, boron and molybdenum application under pot conditions. Spanish Journal of Agricultural Research. 8(3): 797-807.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. Annual Review on Plant Biology. 59: 225-251.