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EFFECT OF AG-NANOPARTICLE IN BIOCHEMICAL AND PHYSIOLOGICAL CHANGES DURING PROGRESS OF SEED DEVELOPMENT AND MATURATION IN GREEN GRAM

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ABSTRACT:

Seed development and maturation comprise a series of events involving cell division/ histo-differentiation, retains deposition, and desiccation. Development of green gram seed has been divided into eight consecutive stages; 5, 10, 15, 20, 25, 30, 35, and 40 days after anthesis (DAA). During seed development, chlorophyll content declined, while carbohydrate and protein contents were increased, suggesting their supportive role in germination and early seedling growth. Only the latter two stages of development (35 and 40 DAA) were taken into consideration for germination potential and seedling metrics, such as vigor index, because seeds produced up to 30 days after anthesis did not germinate at all. The highest germination potential was found in seeds produced at stage 40 DAA in the first and second years, respectively, at 81.24 % and 80.99 %. The peak vigor was noted at 40 DAA, when the average of all genotypes and treatments was calculated. When harvested at 40 DAA, the "Samrat" variety seeds that were grown following seed treatment with Silver nanoparticles (Ag-NPs) had the highest vigor status. Therefore, the best time to harvest green grams for seed is 40 DAA since that is when seed vigor reaches its peak.

Keywords: Ag-nanoparticle, carbohydrate, germination, green gram, protein, vigor, seed development

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1. INTRODUCTION

Seed development within the life cycle of any higher plant may be a crucial process by providing the link between two different sporophytic generations (Baud *et al.,* 2002). Generally, seed development involves an orchestrated program of pattern, which includes formation, reserves deposition and desiccation. A recurrent organic process determines the seed architecture since the formation of a seed is began after double fertilisation happens in an embryo sac (Baud *et al.,* 2002). Subsequently, the phase known as seed development or seed filling commences, during which the water content decreases, storage reserves build-up, and cell expansion occurs. Carbohydrates and storage proteins are chief storage reserves generally accumulated in most of the seeds. Weber *et al.* (2005) stated that the process of filling seeds is highly intricate, including numerous genes and diverse enzymes to regulate the storage of each component. The process of seed filling involves the translocation of monomers of various macromolecules, such as fatty acids, amino acids, and monosaccharides, through phloem sieve elements in order to synthesize their long-chain polymers (Weber *et al*., 2005). Subsequently, the seed proceeds into the drying phase of maturation, during which there are size modifications and a significant decrease in moisture content (Berjak and Pammenter, 2008). Recalcitrant seeds have a high water content (30–70%) when they are shed from the mother plant because they do not go through the maturation drying phase (Berjak and Pammenter, 2008).

The process of seed development is genetically programmed, and developing seed converts the precursors of carbon and nitrogen, into stable reserves required later for various processes (Weber *et al.,* 2005). Reactive oxygen species (ROS), which are produced as a byproduct of metabolic reactions during seed development, have been demonstrated to have two distinct roles: they can be cytotoxic (when present in large quantities) or they can aid in development, dormancy breakage, and defense against biotic and abiotic stresses (Berjak and Pammenter, 2008). It is commonly recognized that these reactive oxygen species (ROS) degrade several kinds of macromolecules, pigments, and other biological components through their reactions (Parkhey *et al.,* 2014). Plant cells have tremendous potency to forestall themselves from such oxidative injury by keeping the reactive oxygen species (ROS) in restraint via an efficient and highly redundant network of antioxidants (Chandra *et al.,* 2015). Enzyme (SOD), catalase (CAT), guaiacol peroxidase (POX), and ascorbate peroxidase (APX) constitute the most important enzymatic systems by which cells catabolize free radicals, thus minimizing the severity of oxidative damage (Chandra *et al.,* 2015). SOD plays a key role in the conversion of superoxide into oxygen and peroxide. While CAT, POX, and APX catalyze the conversion of oxide into water and molecular oxygen and so prevent further generation of free radicals (Chandra *et al.,* 2015). Very limited reports are available concerning the function of antioxidants during seed development however; nobody has yet attempted to explore them in developing green gram seeds.

The physiological and biochemical investigations conducted by He and Gao (2008) and Kok *et al.* (2013) on developing seeds of shrub and *Elaeis guineensis*, respectively, provided comprehensive knowledge regarding seed metabolism. Undoubtedly, knowledge of the biochemical changes that occur throughout development is a necessary foundation for comprehending the regulatory structure that governs the metabolism and development of seeds (Veda and Chakraborti, 2020). Furthermore, a need for any plant-breeding program is a thorough understanding of the mechanisms and pattern of reserve buildup in seeds (Kok *et al*., 2013). Several species' metabolic alterations during seed development have been examined (Baud *et al*., 2002; Silveira *et al*., 2004; Saldivar *et al*., 2011 and Pavithra *et al*., 2014), but there is a complete dearth of information regarding green gram. Green gram seeds are sensitive to desiccation and coldness and hence get damaged. Regarding the metabolic alterations that occur in green gram seeds during their embryonic stages, there is no information available.

Silver nanoparticles (AgNPs) have garnered attention in various fields due to their unique properties, including their antimicrobial, antifungal, and plant growth-promoting activities.

Seed Germination and Root Growth: AgNPs have been shown to positively influence seed germination and root growth (Khan *et al.,* 2023) Treatment of seeds with AgNPs can promote faster and more uniform germination, as well as stimulate root development, leading to stronger and healthier seedlings.

Stimulation of Enzymatic Activity: AgNPs have been shown to stimulate the activity of certain enzymes involved in plant growth and development. This can lead to enhanced photosynthesis, nutrient metabolism, and other essential biochemical processes, ultimately resulting in improved crop productivity (Zhang *et al.,* 2023).

According to the aforementioned perspective of AgNP, the goal of the current experiment was to determine the physiological (germination percentage of seed, seedling length (cm), fresh and dry weight (g) of seedlings; and vigor index) and biochemical (contents of chlorophyll (mg g^{-1}) of whole pod, protein, and carbohydrate of seed) changes during seed developmental stages.

2. MATERIALS AND METHODS

In order to conduct research on seed developmental patterns, three genotypes of green Gram-Pusa Vishal, Samrat, and SML-1822 were sown in 2019 and 2020 at Kalyani D block Farm, BCKV, West Bengal, India. Three distinct genotypes were pre-sown with seed priming made of distilled water and 20 parts per million silver nanoparticles (Chakraborty and Bordolui, 2021). Plant populations grew after distilled water soaking and Ag-NP plots were utilized to assess the influence of Ag-NP on seed development. On a single anthesis date, about 200 flowers from each replication's Ag-NP-treated and distilled water-treated plots were tagged. In the experimental plots with three replications using Randomised Block Design, 20 pods generated from tagged flowers were harvested at 5 days after anthesis (DAA) and then at 5-day intervals until 40 DAA. This resulted in harvesting at 8 different stages of development. The observations on physiological and biochemical characteristics were recorded during different growth stages of the plant. The physiological characteristics like germination percentage of seed, seedling length (cm), fresh and dry weight (g) of seedlings; and vigor index were recorded at 5 days intervals thereafter till 40 DAA. Biochemical characters i.e., chlorophyll content (mg g^{-1}) of the whole pod (Barua *et al*., 2016), soluble protein by Lowry's method (mg g^{-1} fresh weight) (Lowry *et al*., 1951), and total carbohydrate content by Anthrone's method (mg g^{-1} fresh weight) (Hedge *et al*., 1962; Ray and Bordolui, 2022) of seed were observed during the respective developmental stages.

At the first two stages of development stages i.e., at 5 DAA and 10 DAA, protein, and carbohydrate were estimated for an entire pod, as seeds could not be separated well, and were too small and insufficient for analysis irrespective of the genotypes over the years. Only seeds were used for the estimation of both biochemical parameters from 15 DAA to 40 DAA.

3. RESULTS

Variations in the pods' average chlorophyll content (mg g^{-1}) at various post-fertilization phases

Table 1 showed that there were substantial genotype-to-genotype variations in the chlorophyll content of growing pods at different stages of development. Additionally, in both years, there was a significant effect of seed treatment with Ag-NP over the untreated control (Figure 1).

Chlorophyll content was found to be increased at any stages of development over the previous stages till 30 DAA, after which it declined progressively till 40 DAA, which may be due to the stepping towards maturity after attainment at 30 DAA. Average chlorophyll content was estimated as maximum (6.601 and 6.735 mg g^{-1}) of pods irrespective of years for G_1 (Pusa Vishal) followed by that of G_2 (Samrat) and G_3 (SML-1822). Up until 30 DAA, there was a non-significant increase in chlorophyll content for each genotype over the years, regardless of seed treatment, but beyond that, it started to fall.

Variations in the seeds' or pods' average total soluble protein content (mg g^{-1}) at various post-fertilization stages

Total soluble protein content was estimated for whole pods at the first two stages and then it was done for separated developing seeds at all other six stages. Significant variation among the genotypes was recorded for its soluble protein content over the years and it was of the highest value (175.087 and 175.235 mg g^{-1} in 2019 and 2020 respectively) for G_1 (Pusa Vishal) followed by G_2 (Samrat) & G_3 (SML-1822) (Table 2). Pre-sowing seed treatment with Ag-NP was noted to exert a significant influence on the average protein content of seed could be noticed over untreated control in both the years. Average protein content estimated at different stages of development was also found to vary significantly; enhancement in protein content could be recorded till 35 DAA in both years and then declined at 40 DAA (Figure 2), which may be due to deterioration of this sensible biochemical parameter. The soluble protein content of the seed enhanced at an increasing rate till 30 DAA from 15 DAA then the rate of enhancement declined drastically at 35 DAA from 30 DAA. Performance of individual genotypes over stages of development, when the average was made over treatments, exhibited that protein content significantly enhanced with increasing rate till 30 DAA irrespective of the genotypes in both the years, then enhancement occurred at a slower rate from 30 to 35 DAA after which it declined; varying rate of enhancement could be noticed among the genotypes over the years. Whether or not pre-sowing seed treatment was applied, increases in protein content were only observed up to 35 DAA in both years when the average of the genotypes was calculated.

Variations in the mean carbohydrate content (mg g^{-1}) at various post-fertilization phases

Complementing the soluble protein content analysis, the carbohydrate content was also assessed from complete pods during the first two phases of development and from individual seeds during subsequent stages. Though non-significant, higher carbohydrate content was estimated for G_1 (Pusa Vishal) followed by G_2 (Samrat) and G_3 (SML-1822) (Table 3). In both years, the total impact of Ag-NP on enhancement was observed relative to the untreated control (Figure 3). The average increase in carbohydrate content was observed up to 30 DAA in both years, after which it decreased during the last two growth stages; this could be the result of deterioration upon reaching physiological maturity. A similar scenario for individual genotypes over seed treatment procedures could be noticed, though non-significant, with the progress in seed development.

Variations in the germination potential and additional quality indicators of developing seeds

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Only the latter two stages of development (35 and 40 DAA) were taken into consideration for germination potential and seedling metrics, including vigor index because seeds grown up to 30 days after anthesis did not germinate at all for the three genotypes examined here. Average germination potential of the developing seeds varied significantly for the stages of development only in both the years; significant variation among the genotypes and between the stages of development could be noticed for average seedling length along with the significant influence of pre-sowing seed treatment with Ag-NP over that of untreated control, the first order interactions i.e., SXG, SXT and GXT also varied significantly; for average seedling fresh weight, significant variation among the genotypes between the stages of development and treatments were observed along with the first order interactions in both the years; while for seedling dry weight, average genotypic performance varied significantly along with the stages of development and treatment effects only; and clear variation among the genotypes and stages of development were found for average vigour index along with significant influence of Ag-NP over untreated control, first order interactions as well as second order interactions were also significant for this important determined seed parameter. Significantly greater germination potential could be noticed at 40 DAA over that of 35 DAA in both years, when the average was made over genotypes and treatment; but the same could not satisfy the requirement after the minimum seed certification standard (MSCS) specified for this crop at 35 DAA (Table 4). It was of the highest magnitude for G_2 (Samrat) over the other two genotypes, though non-significant. The average maximum germination potential for seeds produced at the stage of 40 DAA in the first and second years, respectively, was found to be 81.24% and 80.99%, both of which were higher than the value given in the MSCS for this crop.

Significantly longest seedlings were recovered for G_2 (Samrat) in both years, on average followed by G_1 (Pusa Vishal) and G_3 (SML-1822) (Table 5). It was of almost similar length for seeds developed at 40 DAA over the years and greater than that of seeds developed at 35 DAA. Significant influence of pre-sowing seed treatment with Ag-NP over untreated control was also noticed in both years. When the average was made over treatments, it was significantly longer for seeds developed at 40 DAA for G_2 (Samrat) followed by that of G_1 (Pusa Vishal) developed at 40 DAA. It was also observed that in both years, seeds formed following Ag-NP seed treatment for certain genotypes produced longer seedlings than their counterparts produced following untreated control.

It could be revealed from both Tables 6 and 7 that both average fresh and dry weight of seedlings were recorded as maximum for G_2 (Samrat) followed by that of G_1 (Pusa Vishal) and G_3 (SML-1822) in both the years; both the parameters were of significantly higher magnitudes for seeds developed at 40 DAA than those of seeds produced at 35 DAA in both the years; and significant influence of pre-sowing seed treatment with Ag-NP, on an average, could be noticed over untreated control. Significant influence of Ag-NP over untreated control could also be noticed for individual genotypes, when the average was made over stages of development, in both years. On average made over treatments, seed produced for G_2 (Samrat) at 40 DAA exhibited significantly higher magnitudes of both fresh and dry weight of seedlings irrespective of the year of study.

Seeds produced from G_2 (Samrat) exhibited significantly highest vigour potential, on average, irrespective of the years followed by that of G_1 (Pusa Vishal) and G_3 (SML-1822). It was of significantly higher magnitude for seeds developed at 40 DAA than that of seeds produced at 35 DAA, when the average was made over genotypes and treatments. A clear significant influence of pre-sowing seed treatment with Ag-NP over untreated control could be noticed over the years, on average; a similar trend could also be noticed for the vigor status of seeds of individual genotypes produced after treatment with Ag-NP in both years. Seeds developed at 40 DAA were of higher vigor potential for individual genotypes, when average as made over treatment (Table 8). It should be observed that the seeds of G2 (Samrat), which were created following seed treatment with Ag-NP, had the highest vigour status when harvested at 40 DAA. These were followed by the seeds of G1 (Pusa Vishal), which were also produced following Ag-NP, while the seeds of G3 (SML-1822), which were produced from untreated control, had the lowest vigour status. Their rigorous genetic potential may be the cause of the variation in seed vigour status among these three genotypes, regardless of the seed treatment and research year.

3. DISCUSSION

Chlorophyll content increased up to 30 DAA but then it decreased until 40 DAA. The same pattern was recorded by Pandita and Nagarajan (2006). They found a rapid decline in chlorophyll content with the advancement of onion seed development. Razzaq *et al.* (2016) studied the effect of Ag-NPs on wheat and found that it has the potential to significantly increase chlorophyll contents. Seed protein content may decrease when it reaches maturity because of denaturation brought on by moisture content release and desiccation.Varied trends in protein accumulation of individual genotypes over the years may indicate strict expression of the inherent potential for this character. Bollini and Chrispeels (1979) reported that a decrease in protein synthesis at maturity in pea and bean (*Phaseolus vulgaris*) seeds was due to the limited availability of mRNA. Krishnaraj *et al.* (2012) found that Ag-NPs showed a significant effect on the synthesis of protein and carbohydrate contents of *Bacopa monnieri*. In case of carbohydrate content, Mehmood and Murtaza (2017) in *Pisum sativum*, found that seed carbohydrate contents were increased after the application of Ag-NPs. Latif *et al.* (2017) reported that the effect of Ag-NPs on the chemical attributes of *Triticum aestivum* significantly increased chlorophyll, carbohydrate, and protein content. Prażak *et al.* (2020) reported that concentrations of Ag-NPs had an immediate beneficial effect, resulting in fast and uniform germination in the laboratory as well as a positive effect in the later stages of seedling development, manifested as an increase in the average seedling height, fresh and dry weight.

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4. CONCLUSION

Pre-sowing seed treatment with Ag-NP significantly enhanced all the parameters over untreated control. The chlorophyll content of developing pods varied significantly among the genotypes and for different stages of development as well a significant influence of seed treatment with Ag-NP over untreated control was also noticed. Chlorophyll content was increased between two consecutive stages of development till 30 DAA, after which it declined progressively till 40 DAA. Seed priming with Ag-NP exerted a significant influence on the average protein content of the seed could be noticed over untreated control. Enhancement in protein content could be recorded till 35 DAA and then declined at 40 DAA. Carbohydrate enhancement could be recorded till 30 DAA and then it was declined at the other two developmental stages. As seeds developed up to 30 days after anthesis did not germinate at all, germination potential and seedling parameters including vigor index were considered for the last two stages of development (35 and 40 DAA) only. The average maximum germination potential for seeds produced at stage 40 DAA in the first and second years was found to be 81.24 % and 80.99 %, respectively. When the average of all genotypes and treatments was calculated, the vigour of the seeds formed at 40 DAA was considerably greater than that of the seeds produced at 35 DAA. When harvested at 40 DAA, the seeds of G2 (Samrat), which were generated following seed treatment with Ag-NP, had the highest vigour status, followed by those of G1 (Pusa Vishal), which were also formed after Ag-NP. The seeds of G3 (SML-1822), which were produced from untreated control, had the lowest vigour status.

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