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Changes in Lipid Profile During Germination of Soybean Seeds (*Glycine max* L. Merr.).

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ABSTRACT

In the present studies, a lipid profile during germination of Soyabean seeds (*Glycine max* (L. Merr.) has been studied. Seeds were treated with different growth regulator including gibberellic acid, phenol, NaCl, abscisic acid, kinetin and indole acetic acid. Total lipids, phospholipids, free fatty acids and total sterols were analyzed at 0, 3, 9, 12, 15 days of germination. The amount of total lipids and phospholipids in treated seeds decreased as compare to control while free fatty acids and sterols increased suggesting that these compounds accelerated the degradation of lipids. The effect of growth regulators namely IAA, GA3, Phenol, Kinetin more or less same whereas a contrast effect for ABA but the extent of effect more or less same. Lipids profile was recorded decrease with increase in days of germination i.e. a little decrease till 3 days which enhanced after 10 days and and become somewhat constant after 15 days.

Keywords: *Glycine max*, lipids, growth regulator, germination, seeds

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INTRODUCTION

Soybean (*Glycine max* (L. Merr.) is a member of family Fabaceae and the world foremost provider of protein and oil. It is often called the miracle crop as it contains high protein content (38–45%) as well as high oil content (20%). Being a globally important commercial crop, grown mainly for its protein, oil and nutraceutical contents each year, soybean provides more protein and vegetable oil than any other cultivated crop in the world. Soybean originated in China, where it has been under cultivation for more than 5000 years.[1] Although grown worldwide for its protein and oil, high value added products such as plant functional nutraceuticals, including phospholipids, saponins, isoflavones, oligosaccharides and human consumption including edible fibre, have gained importance in the last decade. [2,3,4,5]. More recently soybean oil has also been used as an oil source for biodiesel. [6,7]

In a view of above, the soybean seeds have been chosen for the present study with the objective (a) to analyze the changes in lipids contents during germination (b) the effect of growth regulators on protein and lipid contents during germination of soybean seeds. An attempt to prescribe the suitable period for intake of soybean germinating seed vis a vis lipid content.

MATERIALS AND METHODS

Pure line seeds of soybean (*Glycine max* (L. Merr.) var. SL525 was obtained from Punjab Agricultural University, Ludhiana, (Punjab) India. All chemicals used in this study were of analytical grade.

Germination

Seeds were kept for germination in sterile petri plates with moistened filter paper. The effect of biologically active compounds such as gibberellic acid (GA_3, 10^{-5}M), phenol (10^{-5}M), NaCl 10^{-2}M), abscisic acid (ABA, 10^{-5}M), kinetin (10^{-5}M) and indole acetic acid (IAA, 10^{-5}M) was also noted by germinating the seeds in respective compounds separately as did in control. The germination was carried out in dark at room temperature 25°C ± 27°C. The germinated seeds were used for determination of percent seed germination, moisture content, length of radical and plumule and biochemical analysis with an interval of 3 days (0, 3, 6, 9, 12 and 15).

Biochemical studies

For total lipids, phospholipids, free fatty acids and total sterols, one gram of germinating seeds after 0, 3, 9, 12, 15 days were used to carry out biochemical analysis.

For total lipids extraction, germinated seeds was ground in mortar and pestle and were refluxed with 150ml of petroleum ether (60°C -80°C) for 3hr. Supernatant was collected in weighed 100ml flask. Residue was again refluxed with 50ml of petroleum ether for one and half hour and supernatant pooled in the same flask and was evaporated to dryness on hot plate. Increase in the weight of flask determines the total lipids. The residue was dissolved in 25ml of chloroform -methanol (2:1) and stored in -20°C aliquots of which were used for analysis of phospholipids, free fatty acids and sterols.

Phospholipids were extracted on the basis of ashing of total lipids with concentrated H_2SO_4 and perchloric acid (70%). [8]

The free fatty acid content is known as acid number or acid value. It is estimated by titrating it against KOH in the presence of phenolphthalein indicator.

Total sterols were estimated by the methods using acetic anhydride which react with concentrated H_2SO_4 to form coloured complex having maximum absorbance at 625nm. [9]

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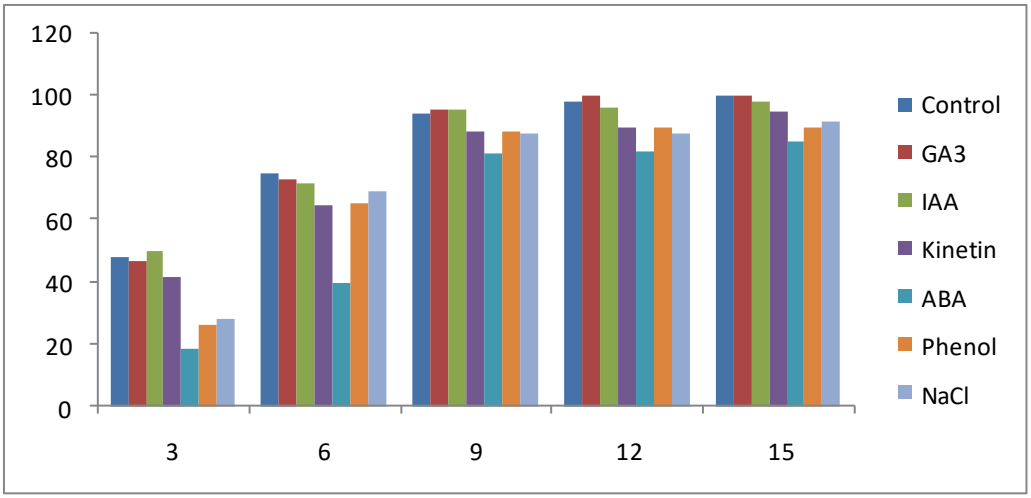
RESULTS AND DISCUSSION

Changes during germination

The rate of germination was observed to be more or less same in water, GA_3 and IAA, whereas, in presence of Kinetin, ABA, NaCl and Phenols, germination decreased during different periods as shown (Graph 1). These confirmed the generally accepted findings where stimulatory role of GA_3 and IAA in cell elongation and cell division have been recorded. The decreased rate in kinetin indicated that either this hormone is not able to reach a specific site in the cells or the endogenous level of this hormone may already be supra optimum so as to mask the effect of exogenous kinetin. [10]

Germination is nearly always inhibited by exogenous ABA is well known fact. Soaking of seeds in ABA presumably, prevents germination by repressing genes or preventing the action of another hormones. [11]

The length of plumule and radicle was more in GA_3 treated seeds as compared to control. IAA also has somewhat promotory action (Graph 1). Similar types of results were reported earlier where authors observed the stimulation of hypocotyls elongation by GA_3 in *Lactuca sativa.* [12] Cell extension in plant tissues is generally regulated by hormones, especially, auxin and gibberellins. ABA decreased the length of seedlings significantly (Graph 1), thus clearly showing its inhibitory effect in seed germination of soybean seeds.



Graphs (1): Percent germination of soybean seeds during different period of germination.

In seed extract

In general, total lipid content decreased from 3rd to 15th day of germination as reported earlier by authors. [13] The lipid content of GA_3 and IAA treated seeds was more or less same on 3rd day while all other treatments showed low values on 3rd day in comparison to control. During 6th to 9th day of germination, lipid content was less in all the treated seeds and on 12th day, it was more in phenols and on 15th day in kinetin in relation to control (graph 2). Similar type of findings have been reported in previous records where changes in seed constituents during germination and seedling growth of precociously matured soybean seeds (*Glycine max*) have been reported. [14]

According to authors, oil and proteins were markedly depleted at 6 days after germination and seedling growth in both precocious and naturally matured seeds. However, the time of lipid mobilization during germination varies in different seeds. A non uniform breakdown of triglycerides has been reported in hazel seeds. In wheat, triglyceride breakdown begin quite early.[15, 16]

In the castor bean however, mobilization of stored lipids begins on about the 3rd day after germination, and digestion is completed within 7 days. By the end of this time, the endosperm is liquefied and

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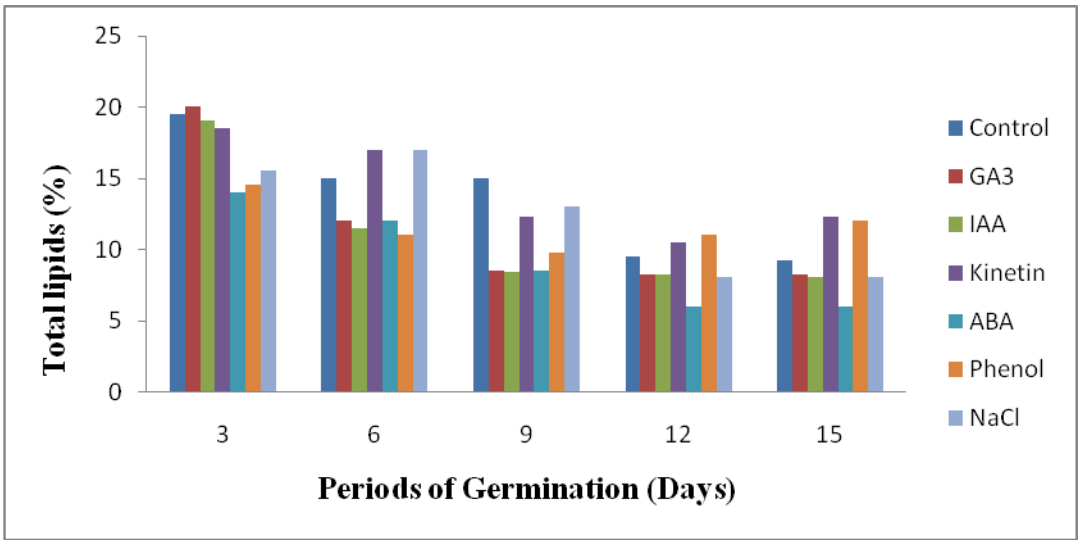
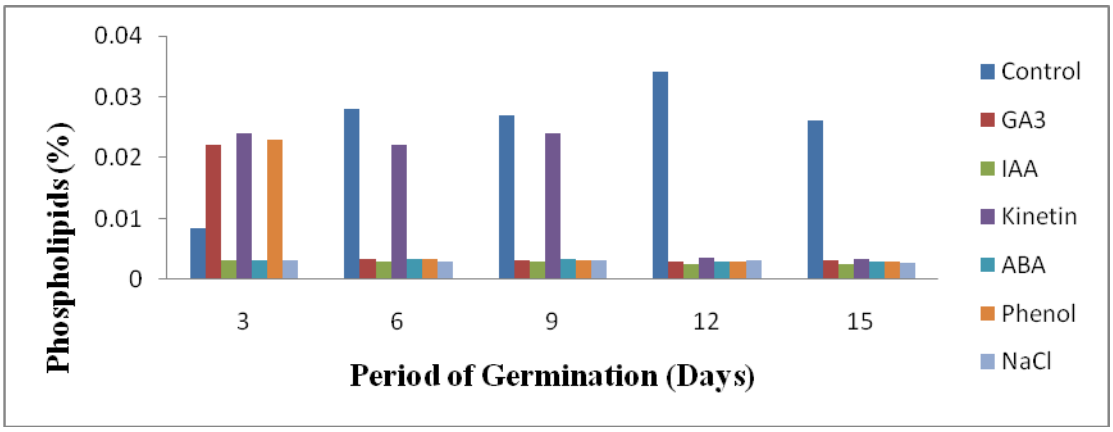
the contents are completely absorbed by the expanding cotyledons, the root-shoot axis having by now grown to about 15cm in length. [17]

The decrease in the lipid content in the soybean seeds during germination in this work reflects that there is degradation of reserve oil to meet the requirement of growing embryo to elicit germination. However, the amount of free fatty acids and sterols also decreased during germination. [18]

It reflects that these all immediately utilized either for the formation of new membranes in germinating soybean seedlings or to meet the energy demand of the growing axis.



Graphs (2): Percent change over control in total lipid content during different periods (3 to 15 days) of germination in soybean.



Graph (3): Percent change over control in phospholipids during different periods (3 to 15 days) of germination in soybean.

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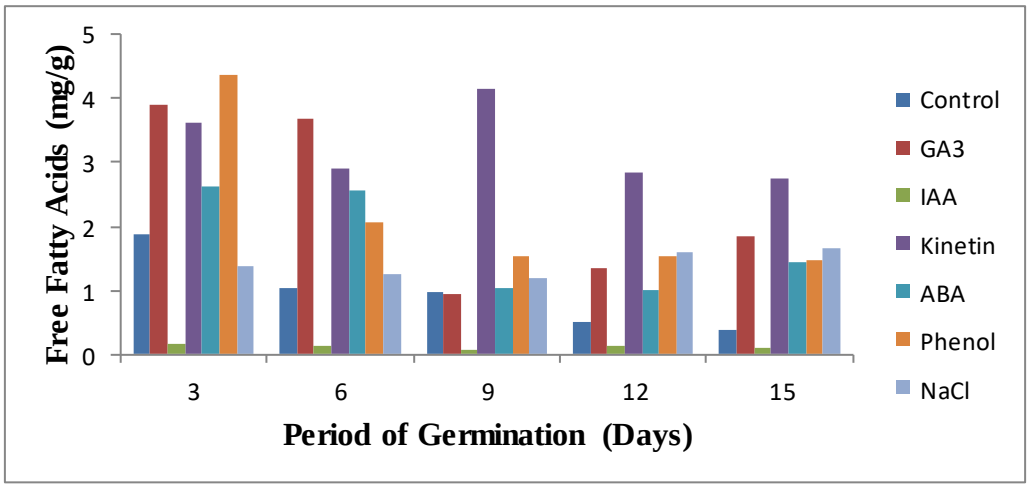
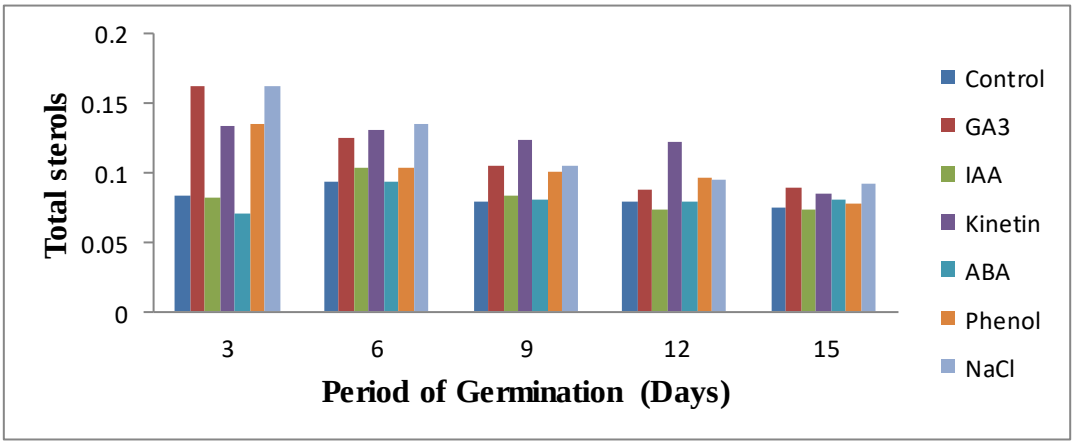


Graph (4): Percent change over control in free fatty acids during different periods (3 to 15 days) of germination in soybean.



Graph (5): Percent change over control in total sterols during different periods (3 to 15 days) of germination in soybean.

In relation to control, phospholipids were less in all the treated seeds **(**Graph 3**)**. Free fatty acids were more in treated seeds except in IAA and NaCl on 3rd day, in IAA on 6th day and 9th day of germination (Graph 4). Total sterols were more in treated seeds at all periods except in IAA and ABA on 3rd day and IAA on 12th and 15th day where these were less than control (Graph 5). Sterols are common constituents of biological membrane and have been shown to influence both structural and functional properties of membranes.[19] Cholesterol is the major sterol present in mammalian cells but the membrane of plant cells contain a number of sterols including campesterol, stigmasterol, sitosterol and sometimes small amount of cholesterol. [20] Sterols and stigmasterols are often the predominant sterol in plant membrane**.** [21] During germination, the amount of these sterols changes to accommodate the reorientation of membrane components.

In the present investigation, the amount of phospholipids, free fatty acids and sterols did not change appreciably during 24hr of imbibition of soybean seeds. As the germination progressed, their content started declining upto 10th day in the cotyledons of cotton seeds, the linoleic and palmitic acid content drops by 50% during 4th day of germination while the stearic and oleic acid content remained unchanged. [22]

One would therefore, suppose that the first step in lipid metabolism is the stepwise, ordered breakdown of triglycerides, certain fatty acids being released preferentially. When the soybean seeds were germinated in GA_3, IAA, kinetin, ABA, Phenol and NaCl, the amount of total lipids and phospholipids become less as compare to control seeds while free fatty acids and sterols increased. It is suggested that these

compounds accelerate the degradation of lipids. Mobilization of lipids is mediated by some important enzymes, and is known to be hormonally controlled. [23]

Plant hormone like GA_3, IAA and cytokinin regulate the metabolic switching. [24] Majority of the findings on hormonal actions are the secretion of metabolites, *de novo* synthesis and secretion of enzymes mostly hydrolases into medium. GA_3 enhances the synthesis and secretion of hydrolases namely, amylases and proteases in aleurone cells of barley. [25] Surprisingly, little information is available on lipase during germination, perhaps because the enzyme is not easily assayed. In wheat, which is not a fatty seed, formation of lipase activity in the endosperm is controlled by the factors emanating from the embryo. [26] Lipase of *Gossypium* cotyledons and acid lipase of *Pyrus malus* (Apple) show small increase in response to applied GA_3.[27,28] The rising activity in acid lipase of *Pyrus* seems simply to coincide with visible germination and growth of the embryos, thus casting doubt on any direct action of GA upon enzyme level.

GA_3 has been shown to cause a marked stimulation in the activity of enzymes of glyoxylate cycle in germinating seeds. In dormant hazel cotyledons, GA_3 causes a 9- fold increase in the specific activity of isocitrate lyase over water incubated tissue. [29] Similarly, GA_3 causes an increase in the activity of isocitrate lyase in the embryos of dormant almond seed. [30] The marked stimulation of the enzymes of the glyoxylate cycle such as isocitrate lyase, certain mitochondrial marker enzymes (cytochrome-oxidase), enzyme of the lipid breakdown and glycolytic enzymes (fructose-I,6-diphosphatase) has been observed in germinating castor seeds in the presence of GA_3.[30]

The direct role of the other hormones in changing the enzymes of lipid metabolism is not known. The interactions of various hormones indicate that they have the potential to regulate germination in numerous ways. The general mechanism of hormone action appears to be as switching agents, shifting one physiologic state to some other physiologic state either by differential transcription or activation of translation, or by alteration of membrane permeability of cells. In general, gibberellins appear to be involved in transcription, kinetins in translation and auxins in membrane permeability.

CONCLUSIONS

It was concluded that the lipids are decreased during germination of seeds and growth regulators observed to accelerate the process notwithstanding the contrasting effect of ABA and Nacl. This lipid breakdown facilitated its mobilization to developing embryo for nutrition. Moreover the recorded value of decreased lipids during germination suggested that the seeds used during germinating are beneficial being little in fat content and alternative of healthy diet.

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