International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

In Vitro Pollen Germination - A Review

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Abstract: ***Pollen is a unique plant tissue that potentially can be used and manipulated to the advantage of the geneticist, breeder, physiologist, or germplasm curator. Since pollen of most species is haploid and each cell is independent, pollen has the potential to provide a microbial-like system for evaluation, assay, and selection. Whatever the interest and use, the viability of the pollen is necessary information. The ability of pollen to germinate on artificial media is widely used as a test of viability, especially for bicellular pollen. This requires a near-optimum germination medium and environment. Adequate media now exist for bicellular pollen from many species. Plant Growth regulators released as secondary metabolites by applied hormones may contribute to the growth promoting effects that enhanced early emergence of pollen tubes. The effect of either sucrose or boric acid individually showed good results, but sucrose in combination with boric acid promoted pollen germination as well as tube development, because boron makes a complex with sugar and this sugar-borate complex is known to be capable of better translocation than non-borate, non-ionized sugar molecules.***

Keywords: Pollen, Pollen germination, Sugar, Boron, Ethylene, temperature

1. Introduction

Pollen is anatomically simple compared to other highly differentiated tissues and plant organs. The pollen grain used to transport the male gamete to the female part of a flower, plays a vital role in breeding programme and hence assists in successful fruit-set. High crop yield generally depends on viable pollen grains. Pollen fertility and viability have a paramount importance in hybridization programme. A large numbers of pollens have been successfully germinated under laboratory conditions on relatively simple media. Pollen performance in terms of germinating ability may have the relative importance upon not only fruit-set but also the flower-flower and flower-pollinator interaction. Pollen germination and pollen tube growth are generally divided into four phases; imbibition phase, lag phase, tube initiation phase and rapid tube elongation phase Pollen tube growth proceeds through tip extension and can be affected by many factors, including temperature, medium osmolarity and the availability of sucrose, calcium, zinc and boron [1], [2]. Calcium is required for maintenance of membrane integrity [3], [4], [5], and increasing evidence suggests that boron plays an important role in the growth and development of vascular plants [6], [7].

2. Literature Surveyed

Germination requirements of pollen vary appreciably from species to species. Apart from moisture, they generally require a carbohydrate source, boron and calcium for satisfactory germination and tube growth. Pollen grains contain different biochemicals like sugar, starch, lipids, phytic acid [8], [9] and m-RNA [9]. These storage products get metabolized on germination and elongation of pollen tube, thus play an important role in germination and in initial stage of pollen tube growth [10], [9].

Sucrose is generally considered to serve two functions in the medium, that of an energy source and an osmoticum. [11] stated that the externally supplied sucrose maintains the osmotic pressure and acts as a substrate for pollen metabolism. [12], [13] recorded that for optimal germination of pollen grains 7.5-20% sucrose solution was needed in different cucurbits depending upon species. Evidence is abundant that sucrose in the medium is metabolized by germinating pollen [14]. For example 10% sucrose in *Bambusa vulgaris* [15] and *Datura metel* L. [16] and *Najas marina* [17] and 15% sucrose in *Bassia latifolia* [18], 11 to 15 % in *Asclepias syrica* [19], 20% in *Abelmnoshus esculentus* [20], 30 % *Catharanthus roseus* [21], [22] proved good for optimum germination. 15% sucrose in water chestnut [23], 5% in selix species [24] proved good for optimum germination.

Pollen germination and tube growth were increased with increasing concentration (0.5-1.0 ppm) of Boric acid, Gibberellic acid, IAA in a basic sucrose and agar medium, without injurious effect. At the physiological level, Boron is believed to control growth, membrane permeability and help in translocation of sugar, while at the biochemical level it controls several enzymes [25]. Several studies have examined the impact of boron on development of reproductive organs [26] Because pollen tubes represent a fast growing system and are sensitive to boron deficiency [27], the morphological effects of boron during pollen tube growth in angiosperms have also been investigated [28].

Boron is an essential microelement required for growth and development of vascular plants. Boron is believed to promote pollen germination by affecting H^+-ATPase activity, which initiates pollen germination and tube growth [27]. Boron deficiency symptoms first appear at growing points, such as root tips and pollen tube tips [29]. Boron deficiency greatly reduces cell wall plastic extensibility and impairs normal cell elongation in growing plant tissue [30]. Boron deficiency also caused morphological abnormalities, including swelling at the tip of the pollen tube. Similar findings have been reported for pollen tubes in several angiosperm species [31]. The role of boron has been confirmed in germinating pollen and growing pollen tubes in vascular plants [32]. The studies of [33] indicated that boron is directly involved in pectin synthesis and thus indirectly involved in development of pollen tube membrane. [34] studied the effect of boron on the localization of pectins and callose in the wall of pollen tubes in *Picea meyeri*. [13] also reported the stimulatory effect of boron on *in vitro* pollen germination of *Pistacia vera*.

The beneficial effect of boric acid or borax in minute quantity on pollen germination was proved my many scientists [35]. They recorded a higher germination percentage and greater length of pollen tube in boric acid containing media than in boric acid free media. Their presence reduced the number of tube bursts. In 0.01% boric acid was standard for *Zea mays* [36]. Higher concentrations were toxic and inhibited germination of pollen as well as growth of pollen tubes [10]. 0.01% Boric acid was also standard for *Brassica oleracea* with 500 µm tube length and for *Chrysanthemum* [37]. 0.001% boric acid gave optimum result in *Sorghum bicolor* L. *Moench*, [23], 0.04% in *Datura metel* L. [16]. 0.01 M boric acid is required in *Conospermum* sp. [38] while 0.01% is required is *Picea meyeri* [39], 0.001 % Boric acid was used with fresh willow pollen [24].

The effect of either sucrose or boric acid individually showed good results, but sucrose in combination with boric acid promoted pollen germination as well as tube development, because boron makes a complex with sugar and this sugar-borate complex is known to be capable of better translocation than non-borate, non-ionized sugar molecules [32].

[40] has shown that calcium plays a role in controlling the permeability of pollen tube membrane. Besides sugar and boron several other substances are also known to have a stimulatory effect on the germination of pollens and the growth of tubes. It is known that K^+ is required for both pollen germination and pollen tube growth [41]. Colchicine, IAA and manganese sulphate in higher concentration inhibits growth of pollen tube [42].

0.03% concentration of $CaCl_2$ proved to be best the best for *Plumeria alba* [43] and *Zea mays* [44] and *Catharanthus* 0.04% concentration of $CaCl_2$ was also best for *Gossypium hirsutum* [22] and *Datura metel* L. [16]. 1.27 mM Ca $(NO_3)_2.4H_2O$ for tobacco (*Nicotiana tabacum*) pollen [45] 250 mg Ca $(NO_3)_2$ required for ground nut i.e. *Arachis hypogaea* [46], 2.12 mM Calcium nitrate for *Sorghum* [46] and 300 ppm Ca $(NO_3)_2.4H_2O$ for *Eucalyptus marginate* plant.

In *Catharanthus* 0.4% $MgSO_4$ gives an excellent result [21], In *Nicotiana tabacum* L. 0.87 mM $MgSO_4.7H_2O$ is required [45], 0.42 mM $MgSO_4.7H_2O$ required in *Gossypium* sp. [47]. 0.001% of Potassium nitrate was also incorporated in the standard media of *Gossypium hirsutum* [48].

Maintaining the germination capacity of stored pollen can be useful in time saving in hybridization programs and also in crops improvement. [49] reported germination of Olive pollen improved markedly in storage conditions. According to [50] the germination capacity of strawberry pollen increased in low temperature. The pollen are of two types Trinucleate and Binucleate, the former one is very hard to germinate on artificial media while the later one is easy to handle. At low temperature the pollen shows germination capacity better than at high temperature. There are several reports on pollen germination and viability of different taxa with varied aims and objective Recently extensive studies have been carried out on pollen storage, various methods have been tried for successful storage of different taxa [51], [24], [52], [53], [54], [55].

The apparent inability of pollen to synthesize ethylene and its insensitivity to exogenous ethylene are unique. Pollen is a rich source of auxin which induces ethylene synthesis in stylar tissue of orchids, and eventually the other floral parts, causing them to senesce [56]. Auxin stimulation of ethylene synthesis in vegetative tissue and fruits is a general phenomenon resulting in many diverse morphological and physiological changes. [57] reported that ethylene increased pollen germination only in the absence of boron in the medium. [58] reports that ethylene increases pollen germination and tube growth.

Pollen grains are rich in hormones, vitamins and amino acids [14]. They generally do not require exogenous supply of these substances for germination and tube growth. There are a few reports in which auxins, gibberellins and cytokinins promote germination and tube growth [60]. Absence of calcium in the medium results in an increase in the membrane permeability leading to the loss of internal metabolites [20]. It is known that pollen germination and tube growth are significantly regulated by the transport of inorganic ions, such as Ca^{+2} and K^+, across the plasma membranes of pollen and/or pollen tubes [2]. KNO_3 may also regulate the osmotic potential for the swelling of pollen grains in poaceae plants [60].

3. Conclusion

Near similarity in the osmotic concentration of the nutrient medium and that of the pollen is a pre-requisite for germination and the percentage of germination and pollen tube length are directly proportional to osmotic concentration, while bursting is inversely proportional [61]. Sugar in the culture medium serves functions like maintaining osmotic pressure of the medium and as a substrate for metabolism of pollen. Satisfactory pollen germination requires solution of sugar, especially sucrose [21], [20] with or without other substances. In most of the plant species tested sucrose is probably the best carbon source both for pollen germination and tube growth followed by glucose, fructose, galactose and lactose [62]. It was generally presumed that sucrose is exogenously hydrolysed by the invertase released by pollen and only hydrolysed products are taken up by pollen [63]. Boron reduces bursting of pollen tube [64] and stimulates pollen germination and pollen tube growth in higher plants [65]. Many other boron containing compounds such as butyl borate, borax, and potassium tetraborate are also effective [66].

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