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Original articles

Effect of seed size on *in vitro* seed germination, seedling growth, embryogenic callus induction and plantlet regeneration from embryo of maize (*Zea mays* L.) seed

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Abstract

Immature embryo-derived callus is more efficient for plant regeneration in maize but appears difficult to obtain in all seasons of the year compared to mature embryos from dry seeds which are readily available throughout the year. This study investigated the effect of seed size on *in vitro* seed germination, seedling growth, callus induction and plantlet regeneration, as well as the relationships between these parameters in five maize varieties. Seeds were designated either as large or small for each variety based on its 100-seed weights, while seed germination were obtained in petri-dishes placed between two sheets of pre-wetted filter paper. Seeds were disinfected, and mature embryos were excised from the maize endosperm and inoculated on the Murashige and Skoog salt (MS medium) supplemented with 30 g/l sucrose, 8 g/l agar, 0.1 g/l myo-inositol and 3 mg/l 2,4-D for callus induction, while embryogenic calli were transferred to medium containing 0.5 mg/l Benzylaminopurine (BAP) and 0.5 mg/l Kinetin for plant regeneration. The study showed that large seed size had significant effect on almost all the traits studied, while positive and significant correlations were observed between *in vitro* germination, seedling growth, callus induction and plantlet regeneration. It can be concluded that callus fresh weight may be used as a marker for improving regeneration efficiency in maize. The results from this study suggest that genetic control of *in vitro* regeneration from maize mature embryo can be utilized to determine inherent genotypic potentials of maize varieties with tissue culture traits for maize improvement.

Keywords: Maize; Seed size; Callus induction; Germination; Plant regeneration; MS medium

1. Introduction

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice in terms of production in the world [9]. It is a major cereal crop for livestock feed, human nutrition and important raw material for several agro-based industries in Nigeria [1]. But under the pressures exerted by limited land, expanding population, plant diseases and insect pest stresses, traditional breeding methods alone have not incorporated the great demand for maize of both quality and quantity. Consequently, several biotechnology approaches have received more emphasis. Among such are particle bombardment [14] and Agrobacterium-mediated [23]. However, success or failure of maize genetic transformation largely depends on the ability of transformed tissues to proliferate and subsequently to regenerate into whole plants.

Immature embryo-derived calli are more efficient for plant regeneration but its production is a time-dependent procedure and difficult to obtain all seasons of the year, while mature embryos from dry seeds are available any time throughout the year. As explants, mature embryos have been used to induce callus and regenerate plants [8]. It has been established that large seeds had higher germination rate, seedling emergence success and more rapid growth than small seeds [21]. The

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higher seedling emergence and growth of large seeds were attributed to large storage reserves in their endosperm or cotyledons and also to their biochemical compositions [13].

Seed size has a special role in crop production. There have been immense studies on seed size in various plant species. Seed size is one of the most important characteristics of seeds that can affect the seed development [19]. Larger seed size indicates a higher protein synthesizing ability, which is probably attributed to more available substrate and energy (ATP), active enzymes and machinery for protein synthesis [6]. Therefore, in endosperm-supported mature embryo culture, seed size, which is proportionately reflected in the endosperm size, may also affect callus induction and plant regeneration [17].

Seed size has also been reported to have effect on tissue culture response of callus from endosperm-supported mature embryos in barley [17], wheat [20] and rye [24] while little information is available on the effect of seed size on *in vitro* seed germination, seedlings growth, embryogenic callus induction and plantlets regeneration from mature embryos in maize. This study therefore sought to determine the effect of seed size on *in vitro* seed germination, seedling growth, callus formation, plantlet regeneration as well as the relationship among these parameters.

2. Materials and method

The experiment was conducted at Biotechnology laboratory of Institute of Agricultural Research and Training (I.A.R&T), Ibadan, Oyo State in 2015. Physiologically matured and well dried cobs of five maize varieties (Table 1) were obtained from seed production field of I.A.R&T., Ibadan. The cobs were shelled and seeds were designated as large or small according to seed weight (g). Seeds weighing less than 25 g were grouped as small seed, while seed weighing above 25 g were classified as large seed (Fig. 1). Most large seeds were shelled from the bottom of the cobs while small seeds were shelled from top of the cobs. One hundred small and large seeds of each variety in four replicates were weighed to determine the 100 seed weight. Mean of the four replicates were recorded (Table 1). Ten seeds were randomly selected from each category to determine seed morphometric parameters through the use of digital Vernier calliper (Table 2).

2.1. In vitro seed germination

The seeds were washed with Tween20 (detergent) under running tap water. They were then disinfected in 70% methylated spirit for 5 min and rinsed in three changes of sterile

Table 1
Grouping of maize seeds based on their 100-seed weight (g).

Maize varieties	Small seed size	Large seed size
DMR-LSR-Y	23.93	27.77
BR9943DMR	24.09	29.75
ART/98/SW6-OB	24.19	28.86
SUWAN-1-SR-Y	23.69	29.69
DMR-ESR-Y	22.29	28.36

Fig. 1. Seed sizes of five maize varieties; **A**: Small seed size; **B**: Large seed size; **1**: DMR-LSR-Y; **2**: BR9943DMR; **3**: ART/98/SW6-OB; **4**: SUWAN-1-SR-Y; **5**: DMR-ESR-Y.