Picloram levels in the responses of oil palm (Elaeis guineensis) zygotic embryo in vitro


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Abstract

The auxin, 4-amino-3,5,6-trichloropicolinic acid (picloram), is a strong alternative to the widely used 2,4-dichlorophenoxyacetic acid (2,4-D) and plays a vital role in plant regeneration processes. This study was undertaken to investigate the effect of picloram in the morphogenic responses of oil palm zygotic embryo explants. Immature zygotic embryos were sterilized, excised and cultured on Murashige and Skoog (MS) medium supplemented with picloram at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5mg/l concentrations. After four weeks in culture, 80-100% of the explants had increased sizes (swelling) in all treatments. At six weeks, shoots were observed in cultures containing picloram at 0, 0.5, 1.0 and 1.5mg/l concentrations. There was 40% shoot presence at 0.5 and 1.0mg/l concentrations. In the eighth week of culture, somatic embryos were generated in 10%, while roots were formed in 20% of the cultures without growth regulator. There was also 20% callus proliferation in the cultures supplemented with 1.5mg/l concentration. Picloram concentrations of 2.5, 3.0 and 3.5mg/l had no effect on zygotic embryos in culture for the duration of this study. The results obtained indicated that in vitro morphogenetic responses of oil palm zygotic embryo could be by picloram concentration of 0mg/l for direct somatic embryogenesis and/or root formation. There was also the indication that 0.5 and 1.0mg/l may be for shoot generation and 1.5 mg/l for callus induction.

Keywords: Oil palm, Picloram, Morphogenesis, Zygotic embryo, in vitro

Introduction

Oilpalm (Elaeis guineensis Jacq.), a major source of edible oil in the world is a key resource especially in the vegetable and biofuel industries. It is a perennial and open-pollinated plant whose breeding cycle usually takes more than ten years [1]. In nature there are three varietal types of oil palm which have been classified on the basis of the presence or absence of a shell in fruit. Dura produces fruits with a thick shell, Pisifera which is without a shell in its rare fruits and Tenera which is a hybrid cross of these two variety types and produces fruits with an intermediate shell [2]. The most commercial oil palm are high yielding Tenera varieties. However, this cultivar typically shows considerable variation in oil yield and other agronomically important traits [3]. Globally, it seems difficult to obtain a required amount of high yielding, uniform Tenera variety. Oil palm clones offers the potential for higher yield/productivity because it is possible to establish uniform trees comprised of identical clones of highly productive genotypes [4]. Commercial propagation of oil palm through tissue culture is therefore widely used. Plant regeneration of oil palm through culture in vitro has been reported by several researchers [5, 6]. A reliable and efficient procedure for propagation of elite in vitro clones has the potential to increase yields significantly. Oil palm tissue culture procedure has undergone several improvements in recent years. A range of explant source has been used to establish tissues in culture. These include zygotic embryos, inflorescence, roots and young leaves. The response of plant tissues in culture is influenced mainly by genotype and donor plant age [7], in addition to the influence of the appropriate media, plant growth regulators and explants developmental stages [8] are also important. Explants of young tissues still undergoing cell division generally form callus more readily than those from older parts of plant, where the meristematic cell activities are reduced or lost. A juvenile explant is a prerequisite in non-conventional method of plant genetic manipulation and as such, zygotic embryos are commonly used [9]. Zygotic embryos have been used to generate callus in previous oil palm tissue culture studies [10, 11]. The basal media used by these researchers where supplemented by auxins. An embryo callus possesses a better regenerative ability compared to those derived from mature organs such as leaf, stem and root [12]. Plant embryo culture is an invaluable breeding technique since it offers possibilities to synthesize hybrids from incompatible crosses [13]. The influence of plant growth regulators in the direction of tissues in vitro is dependent on the type and concentration of phytohormone present in the media. Auxins such as Picloram, Naphthalene acetic acid (NAA), Indole acetic acid (IAA) and 2,4dichlorophenoxyl acetic acid (2,4-D)have been used to establish in vitro cultures of some plant species [14, 15]. Picloram is a strong alternative to the widely used 2,4-D [12]. The aim of this study was an attempt at investigating the response of oil palm zygotic embryo in culture containing varied picloram concentrations.

Materials and Methods

This study was carried out using the facilities of Tissue Culture Laboratory of the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria.

Plant materials: Oil palm (tenera) seeds of four (4) months old were collected from Seed Production Division of NIFOR. The seed coat was broken with a nut cracker.

Media preparation and phytohormone supplementation: The zygotic embryos were cultured on Murashige and Skoog (MS) medium supplemented with 3% sucrose and various levels of picloram (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5mg/l) the
pH was adjusted to 5.7 and media solidified with 10g/l plant agar.

**Disinfection and excision of kernels:** The kernels were washed using detergents and rinsed under a running tap. They were further rinsed in the laminar flow chamber with distilled water and sterilized for two minutes each in 0.1% mercuric chloride (0.1g in 100ml of distilled water), 70% ethanol and in 3% sodium hypochlorite which contained two drops of tween 20. The kernels were thoroughly rinsed with sterile distilled water between sterilization. It was thereafter rinsed twice with sterile distilled water. Zygotic embryos were excised from the kernel with the aid of sterile blade and forceps were used for inoculation. The cultures were thereafter incubated in the dark at 25°C.

**Experimental design and statistical tool:** The experiment was set up to test a single factor effect. There were eight treatments (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5mg/l). 0mg/l served as the control. Each treatment had twenty replicates. Uniformity was maintained in all supporting materials during observations/data collection. The observations made were classified as binomials (response, present or absent, expressed as percentages).

**Results**
In the course of this study, observations were made on a two week interval from the day of inoculation. The results presented were for duration of eight weeks. On the second week of culture, there was no visible response. At the fourth week, the explants size had increased. 80-100% of the explants were swollen in all treatments. Table 1 shows the percentage increase in sizes. On the sixth week, zygotic embryos in culture with picloram at 0, 0.5, 1.0 and 1.5mg/l were observed to have developed shoots. There was 40% shoot presence in the treatment with 0.5mg/l concentration, while treatment 1.0 and 1.5mg/l had 20% shoot presence. 0mg/l treatment had the least shoot presence of 10% (Table 2).

<table>
<thead>
<tr>
<th>Picloram concentration (mg/l)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100% swelling</td>
</tr>
<tr>
<td>0.5</td>
<td>100% swelling</td>
</tr>
<tr>
<td>1.0</td>
<td>100% swelling</td>
</tr>
<tr>
<td>1.5</td>
<td>90% swelling</td>
</tr>
<tr>
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<td>100% swelling</td>
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<tr>
<td>2.5</td>
<td>90% swelling</td>
</tr>
<tr>
<td>3.0</td>
<td>80% swelling</td>
</tr>
<tr>
<td>3.5</td>
<td>80% swelling</td>
</tr>
</tbody>
</table>

**Table 2:** Effect of different concentrations of picloram on the response of oil palm zygotic embryo at 6 weeks in MS medium.

<table>
<thead>
<tr>
<th>Picloram concentration (mg/l)</th>
<th>Shoot</th>
<th>Root</th>
<th>Callus</th>
<th>Somatic embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>+++++</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>1.0</td>
<td>++</td>
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<td>3.5</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

Keys: - absent; +10% presence; ++ 20% presence; ++++ 40% presence

In the eighth weeks of culture, 10% shoot formation was observed in the cultures supplemented with 2mg/l picloram concentration. 20% callus formation was evident in the 1.5mg/l treatment. Cultures without growth regulator had 20% root formation, 10% shoot and somatic embryo presence (Table 3). The result in figure 1 portrays the responses observed after eight weeks in culture. Throughout the duration of the study, zygotic embryo explants inoculated in media supplemented at the rate of 2.5, 3.0 and 3.5mg/l were irreversible. From the results, it can be deduced that picloram concentration of 0.5mg/l was optimal for shoot formation while 1.5mg/l concentration stimulated callus formation. The introduction of picloram at the various levels under investigation had no effect on root and somatic embryo formation.

**Table 3:** Effect of different concentrations of picloram on the response of oil palm zygotic embryo at week 8 in MS medium

<table>
<thead>
<tr>
<th>Picloram concentration (mg/l)</th>
<th>Shoot</th>
<th>Root</th>
<th>Callus</th>
<th>Somatic embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>++</td>
<td>-</td>
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<tr>
<td>0.5</td>
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<tr>
<td>1.0</td>
<td>++++</td>
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<tr>
<td>3.5</td>
<td>-</td>
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</tbody>
</table>

Keys: - absent; +10% presence; ++ 20% presence; ++++ 40% presence
Fig 1: *In vitro* responses of oil palm zygotic embryo cultured in MS medium

a) Somatic embryo induced from zygotic embryo explant in medium without growth regulator.
b) Shoot generated in medium supplemented with picloram at 0.5mg/l.
c) Callus at base of zygotic embryo explants in medium supplemented with picloram at 1.5mg/l.
d) Root generated in medium without growth regulator.

**Discussion**

The level of auxin supplementation enables diverse responses of explants in *in vitro* studies [16]. This is evident from the results obtained in this study. After four weeks of culture on MS medium supplemented with picloram (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5mg/l) almost all zygotic embryos increased in size. This is similar to the observation of Thuzar and his team [6] in their work on the efficient and rapid plant regeneration of oil palm zygotic embryos. Zygotic embryos are naturally determined for shoot or root formation but may also be manipulated to induce callus or somatic embryos by growth regulators [17]. On the sixth week, there was shoot production from immature zygotic embryo in some treatments. The shoots were induced in MS medium containing 0, 0.5, 1.0 and 1.5mg/l of picloram with the most shoot presence (40%) obtained in the medium supplemented with 0.5mg/l and the least shoot presence (10%) at 0mg/l concentration. The 10% shoot formed at 0mg/l may be as a result of the cells natural ability to regenerate due to cell competence [17]. Possibly the cells’ natural growth substance level at the time of excision enabled it to form shoots in the absence of exogenous growth regulators while the 40% shoot presence in the 0.5mg/l treatment may have been as a result of introduction of growth regulator. The finding is similar to the result of Şener and colleagues [18] in their study of plant regeneration from immature inflorescence of barley. They obtained 40.6% shoot presence as optimal value when growth medium was supplemented with picloram, though at a much higher concentration of 7.5mg/l. In the present study, shoot presence at the sixth week reduced to 20% with an increase in the picloram level from 0.5 to 1.0mg/l. Subsequent increase in picloram levels to 1.5mg/l did not change the percentage of shoot presence as 20% was also obtained.

The results obtained (Table 3, Figure1 {a&d}) provided evidence that somatic embryos and roots can be induced from immature oil palm zygotic embryo without exogenous growth regulator. It may be that the interplay between the cells natural growth substances at the time of excision and the nutritional constituents of the basal medium was sufficient for the induction of roots and somatic embryos. The effect of 1.5mg/l concentration of picloram resulted in 20% callus formation at the base of the zygotic embryo. In a previous study by [19], callogenesis was observed on immature zygotic embryo of peach palm at a rate of 34% in cultures supplemented with 25µm picloram after 41 days. The difference between the time taken for the onset of callogenesis in the present study and in that of Maciel and co-workers could be the difference in the source of explants used in the studies.

**Conclusion**

It can be inferred from the present study that the response of immature zygotic embryo *in vitro* vary depending on the concentration of picloram used.

**Acknowledgement**

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**References**


