Vol. 18(10), pp. 125-133, November, 2024
DOI: 10.5897/JMPR2024.7356
Article Number: A06D1F672784
ISSN 1996-0875

http://www.academicjournals.org/JMPR

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Journal of Medicinal Plants Research

Full Length Research Paper

Development of *in vitro* protocol for large-scale clonal propagation of *Capparis erythrocarpos* for the herbal medicine industry and conservation

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Received 8 July, 2024; Accepted 30 October, 2024

The increased use of *Capparis erythrocarpos* for treating rheumatoid arthritis and as an appetite stimulant has necessitated the establishment of efficient *in vitro* propagation protocols for large-scale production of seedlings to ensure its sustainable use in the herbal medicine industry and conservation of the species. In this study, mechanical scarification and priming of seeds with sulfuric and GA_3 induced only swelling of seeds on Murashige and Skoog (MS) medium supplemented with 0.5 – 2.0 mg/l BAP or kinetin but no shoot development. Shoot tip and nodal cutting explants cultured on MS medium supplemented with 2 g/l of activated charcoal and 0.1 mg/l each of GA_3, NAA and BAP induced callus development. Subsequent transfer to a fresh medium resulted in multiple shoot formation. The results suggest that it is possible to exploit *in vitro* propagation techniques to produce planting materials of *C. erythrocarpos* for sustainable use in the herbal medicine industry as well as conservation of the species.

Key words: *Capparis erythrocarpos,* shoot tips, nodal segments, seeds, callus, *in vitro* propagation, genetic erosion

INTRODUCTION

The *Capparaceae* family, which is distributed widely in the Mediterranean regions (Hall et al., 2002; Inocencio et al., 2006) consists of 350 species, including *Capparis spinosa*, *Capparis orientalis* Veill, *Capparis ovata* and *Capparis erythrocarpos,* which are exploited for medicinal and culinary uses. They are perennial xerophytes with well-developed root systems that help to reduce soil erosion and contribute to the preservation of arid ecosystems due to moderate water requirements (Chedraoui et al., 2017; Gan et al., 2013; Koufan et al., 2022; Zuo et al., 2012). In Ghana, *C. erythrocarpos* grows wild in the lower plains of Tema and Accra in the Greater Accra Region and some parts of the Eastern Region (Kyene et al., 2022). The plant is found across tropical Africa in several countries, notably, Uganda, Kenya, Tanzania and Ethiopia (Geissler et al., 2002 Hedberg et al., 1983; Kisangau et al., 2007). Beyond

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Africa, the plant is also found in India, Sri Lanka and the Himalayas (Ayyanar and Ignacimuthu, 2011).

Members of *Capparaceae* are rich in bioactive compounds, including phenolic acids, flavonoids, alkaloids, and phytosterols, which are highly exploited in the herbal medicine industry (Wojdyło et al., 2019; Zhang and Ma, 2018). Traditionally, *C. erythrocarpos* is used for the management of pain, arthritis, blurred vision, and other forms of inflammatory conditions in several African counties (Adeniyi et al., 2018; Martey et al., 2013). The roots exhibit anti-inflammatory and anti-arthritic activity (Danquah et al., 2021; Twumasi et al., 2019). Additionally, the leaves and stems of the plant have been shown to possess analgesic and anti-arthritic effects, thus, its use in the herbal medicine industry to commercially produce 'Sirrapac', a powdered product used in the treatment of rheumatoid arthritis and appetite stimulants in Ghana (Kumatia et al., 2019; Kyene et al., 2022; Twumasi et al., 2019). Despite its medicinal importance in the herbal industry in Ghana and across Africa, C. *erythrocarpos* still grows in the wild with little or no effort to conserve the species or cultivate it for commercial exploitation. Currently, plant material of C. *erythrocarpos* is harvested from the wild or naturally growing plant populations. However, this practice of the continuous harvest of plant parts from the wild for the production of herbal medicine can lead to local extinction or genetic erosion of C. *erythrocarpos* and other important medicinal plants if sustainable propagation protocols are not developed (Asase, 2023). Furthermore, deforestation of the plant's natural ecosystem, expansion of agricultural land area and climate change variability threaten the productivity and geographical distribution of C. *erythrocarpos* (Applequist et al., 2020; Asase and Peterson, 2019).

Conventionally, the genus *Capparis* is sexually or asexually propagated via seeds or stem cuttings, with seeds being the most commonly used (Koufan et al., 2022). However, sexual propagation via seeds results in low germination due to high dormancy, while its high heterozygosity limits the potential use for large-scale clonal propagation (Gask et al., 2008; Kereša et al., 2019). Although asexual propagation via cuttings provides an alternative, it is also limited by poor rooting of cuttings mainly due to lignification (Bahrani et al., 2008; Kereša et al., 2019). Alternative approaches that overcome the limitations associated with using seeds and cuttings for the propagation of C. *erythrocarpos* will greatly enhance its use for medicine and the conservation of this important species (Koufan et al., 2022; Sottile et al., 2021).

In vitro regeneration or micropropagation offers an alternative approach to overcome the limitations associated with conventional propagation of *C. erythrocarpos* via seed or stem cuttings. It involves the culture of seeds or stem cuttings on an appropriate culture medium under controlled conditions (temperature and light) to promote germination and growth into plantlets (Thorpe, 2012)." The SE system involves the generation of organised embryogenic structures (OES) or proembryo masses that differentiate into somatic embryos giving rise to plantlets under appropriate conditions (Bhatia and Bera, 2015; George et al., 2007; Ikeuchi et al., 2016). The establishment of SE in *C. erythrocarpos* will facilitate the extraction of secondary metabolites from embryogenic callus for medicine use by the pharmaceutical industry. Furthermore, successful *in vitro* protocols for *C. erythrocarpos* will enable the mass production of seedlings to provide sufficient plant material for medicine and conservation of the species.

The successful development of *in vitro* propagation protocols has been reported for *Capparis spinosa, C. orientalis* and *C. repestris* using seed and nodal segment explants (Awatef et al., 2017; Germanà and Chiancone, 2009; Gianguzzi et al., 2019; Saleh et al., 2024). In Tunisian Caper (*Capparis spinosa* L), *in vitro* seed germination and seedling development was successful on Murashige and Skoog (MS) medium after pretreatment of seeds with sulfuric acid and GA_3 (Awatef et al., 2017). Similarly, the pre-treatment of seeds with heat before culture on modified MS medium *in vitro* resulted in over 80% seed germination in *C. rupestris* (Germanà and Chiancone, 2009). However, there are no reports of in vitr *o* propagation protocols for *C. erythrocarpos* despite its economic importance in the herbal medicine industry. The pretreatment of *C. erythrocarpos* seeds with GA_3 or scarification will result in germination *in vitro*. Furthermore, the culture of shoot tips or nodal cuttings on MS medium supplemented with GA_3, auxins or cytokinins will likely stimulate shoot growth and promote whole plant regeneration.

In this study, the first objective was to develop an efficient *in vitro* propagation protocol for the production of planting materials for large-scale cultivation of *C. erythrocarpos* for the herbal medicine industry. The second objective was to develop improved plant regeneration protocols via indirect somatic embryogenesis for the production of totipotent tissues for the genetic improvement of *C. erythrocarpos*.

MATERIALS AND METHODS

Collection of plant materials

In May 2019, fresh and dry fruits were collected from mature *C. erythrocarpos* plants growing in the Greater Accra Region of Ghana, specifically, in the Tema Community 22 area located at 5.718597 N, -0.030503 E. The area has an annual mean temperature of 28.6 - 30.3°C and a mean relative humidity of 77% (Weather and Climate, 2019). The altitude of the area is 34 meters above sea level. The identification of *C. erythrocarpos* plant species was based on an experienced tree spotter familiar with the local flora. Voucher specimens were collected and compared with already identified specimens at the Herbarium of the Center for Plant Medicine Research, Mampong, Ghana to confirm their identification.

Furthermore, key morphological characteristics of the species were confirmed using relevant literature, including the Flora of Ethiopia and Eritrea (Demissew, 2006), Flora of West Tropical Africa (Heine, 1963), and a variety of online taxonomic resources. The fruits were taken to the laboratories of the Biotechnology Center,

Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra, Ghana.

Seeds were extracted from both fresh and dry fruits, washed with water for 5 min to remove debris and air-dried for an hour. Seeds were then stored in a paper envelope at room temperature (ca. 28°C) before use for experiments. The fruits were categorized based on size and weighed to determine the weights. After that, the seeds were extracted from the fruits, washed thoroughly with water and viewed under a stereomicroscope (Motic SMZ-161, China) to study their morphometric characteristics. The seed coat was also removed by careful dissection with a scalpel blade to observe the endosperm under the stereomicroscope.

Pretreatment of seeds for germination

Priming of seeds with sulfuric acid

Both dry and fresh seeds were soaked in 50 ml of water in honey jars for 24 h. The seeds were then scarified by soaking in 90% sulfuric acid (H_2SO_4) for 1 or 2 h on an orbital shaker at 90 rpm. Afterwards, seeds were rinsed in three changes of sterile distilled water (SDW) followed by immersion in 20% bleach (Clorox®, active ingredient – 6.05% sodium hypochlorite) for 20 min to begin sterilization. The seeds were finally surface sterilized by immersion in 70% ethanol for 10 min and rinsed with three changes of SDW under a laminar flow hood (Nuaire, Plymouth, UK). Sterilized seeds were cultured on 25 ml full-strength Murashige and Skoog (1962) basal salts with vitamins (MS) (Duchefa Biochemie, RV Haarlem, Netherlands) similar to earlier work on Caper *in vitro* propagation (Chalak et al., 2003; Germanà and Chiancone, 2009). The MS medium was supplemented with varied concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of 6-benzylaminopurine (BAP) or kinetin (Sigma Aldrich, Munich, Germany) including a control (0 mg/l) in honey jars. The experiment was carried out in a randomized block design with four BAP or kinetin concentrations and two sources of seed as the factors. Ten seeds were inoculated per treatment. The control treatment had no BAP or kinetin. One seed was cultured in a jar and this constituted a replicate. The cultures were observed for germination and recorded.

Priming of seeds with sulfuric acid and gibberellic acid (GA_3)

Fresh seeds harvested from mature fruits of *C. erythrocarpos* plants were washed in soapy water (Morning Fresh Dishwashing Liquid, Cussons®) for 5 min to remove debris. Seeds were then scarified by using a modified protocol reported by Bahrani et al. (2008). Seeds were immersed in 98% H_2SO_4 for 15 min on an orbital shaker at 90 rpm and rinsed thoroughly with three changes of SDW. Seeds were finally soaked in different concentrations (500, 1000, 1500, or 2000 mg/l) of gibberellic acid (GA_3) for 3 or 6 h with only SDW as a control (Awatef et al., 2017; Germanà and Chiancone, 2009). The experiment was conducted in a randomized block design with four GA_3 concentrations and two soaking durations as the factors. Ten seeds were soaked for each GA_3 concentration and each soaking duration. After this pre-treatment, the seeds were sterilized in 10% bleach (Clorox®, active ingredient – 6.05% sodium hypochlorite) for 10 min, rinsed in water and surface sterilized with 70% ethanol for 10 min and rinsed with 3 changes of SDW. Seeds were cultured in liquid or solid full-strength Murashige and Skoog (1962) basal salts with vitamins (MS) without growth hormone. Liquid cultures were kept on an orbital shaker at 75 rpm.

Mechanical scarification of seeds

Dry seeds of *C. erythrocarpos* were mechanically scarified by complete removal of the seed coat using a plier and pre- treated by soaking them in either 1000 mg/l of GA_3 or water (control) for 1 h. The pre-treated seeds were sterilized in 20% bleach (Clorox®, active ingredient – 6.05% sodium hypochlorite) for 10 min, rinsed in SDW, and then surface sterilized with 70% ethanol for 10 min. The seeds were then rinsed with 3 changes of SDW and cultured on half-strength Murashige and Skoog (1962) basal salts with vitamins (MS) supplemented with 1mg/l of GA_3 and 1 or 2 mg/l BAP or 1 or 2 mg/l Kinetin. The use of MS medium supplemented with GA_3 and BAP has been reported to promote seed germination in Caper (Koufan et al., 2022). 10 seeds with or without seed coats were cultured for each treatment using a randomized block design. One seed was cultured per jar and formed a replicate. Successful seed germination was defined as the emergence of a radicle (Leubner-Metzger, 2003).

Shoot tips and nodal cuttings culture

Shoot tips and stem segments were harvested from young seedlings (12 months old) of *C. erythrocarpos*. 2 node cuttings derived from the stem segments and shoot tips were kept under running water for 1 h. Both explants were washed in mild detergent (Morning Fresh Dishwashing Liquid, Cussons®) for 30 min on an orbital shaker at 90 rpm. The explants were then transferred to 0.5% (w/v) fungicide solution (Bendazim® 50WP, Agrimat Limited, active ingredient, carbendazim) for 15 min on the orbital shaker for treatment against systemic fungi infection. The treated explants were rinsed with three changes of SDW and transferred to 100 mg/ml cefotaxime (Sigma Aldrich, Munich, Germany) solution for 15 min on the orbital shaker at 90 rpm for treatment against bacteria. The final surface sterilization of explants was completed by immersion in 70% ethanol for 5 min and rinsing with three changes of SDW under the laminar flow hood (Nuaire, Plymouth, UK). 20 explants were cultured for each treatment using a complete block design with five explants for each replicate. The two treatments used were MS basal medium with vitamins at full strength or half strength supplemented with 0.1 mg/l each of GA^3, BAP and NAA (Chalak and Elbitar, 2006; Koufan et al., 2022). Shoots formed were transferred to MS basal medium with vitamins supplemented with 2 g/l of activated charcoal, and 0.1 mg/l each of GA_3, NAA and BAP. Successful shoot growth was defined as the formation of at least one leaf or leaflet by shoot tips or nodal segments.

Callus induction from leaf explants

Young leaves were harvested from 12-month-old young seedlings of *C. erythrocarpos* for callus induction. Leaf explants were washed thoroughly under running tap water for 5 min before sterilization with 20% bleach (Clorox®, active ingredient – 6.05% sodium hypochlorite) for 10 min. Leaves were then rinsed with three changes of water, surface sterilized with 70% ethanol for 5 min in the laminar flow hood followed by rinsing with three changes of SDW. Sterilized explants were sectioned into square cuts (2 x 2 mm) using a scalpel blade on a sterile tile. Cut leaf explants were cultured on MS basal medium with vitamins supplemented with 1, 2, 3 or 4 mg/l 2,4-D or picloram including controls (Elmaghrabi et al., 2017) and kept in the dark at 25 ± 1 °C for 7 days or 28 days in the growth room. The cultures were monitored and the number of calli formed by leaf explants was scored. Callus formation was scored as successful by the conversion of leaf explants to organised embryogenic structures. The experiment was carried out using a randomized complete block design with four 2,4-D concentrations as the factor. Six cut leaf explants were cultured per jar and replicated five times. After 28 days of culture in the dark, the callus formed was transferred to MS basal salts medium with vitamins supplemented with 0.1 mg/l each of NAA, BAP and GA_3 and kept in light. All cultures were kept at 25 ± 1°C under cool fluorescent light (16/8-h light/dark regime; 2500 lux) in the growth room. Callus cultures were maintained at 25 ± 1°C in the dark

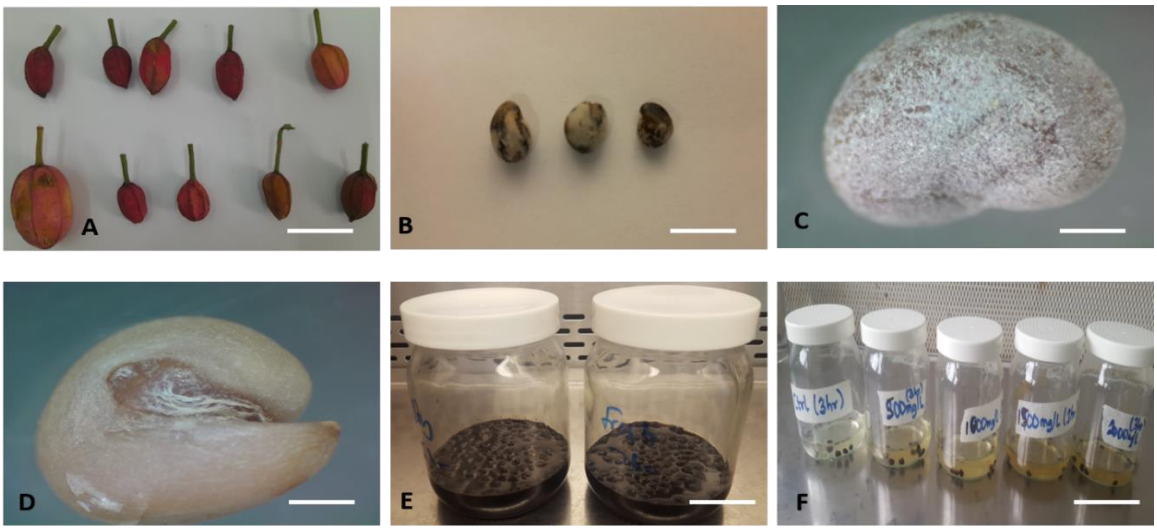

Figure 1. Fruit/seed morphology and dormancy breaking of *C. erythrocarpos* seeds. (A): Fruits of *C. erythrocarpos;* (B): seeds with intact seed coat; (C): covered by mucilaginous layer; (D): without seed coat; pretreatment of seeds using; (E): sulfuric acid; (F): gibberellic acid (GA_3). Bar represents 2 mm (A) and 5 mm (B - F).

for 28 days.

Data analyses

All data collected was analyzed using GraphPad Prism version 7 (San Diego, United States). Data for priming of seeds with varied concentrations of GA_3 was subjected to a two-way analysis of variance (ANOVA). The separation of means was performed using Tukey's multiple comparisons tests at $p \leq 0.05$. Data for mechanical scarification of seeds and regeneration by shoot tips or nodal cuttings was subjected to ANOVA.

RESULTS AND DISCUSSION

Seed and fruit characteristics

The morphology of *C. erythrocarpos* fruits can be described as a berry with an ellipsoid or ovate shape weighing between 3.9 to 48 g and red when ripe (Figure 1A). The seeds are reniform in shape and blackish-brown in colour with a mucilaginous whitish layer around the seed coat (Figure 1B, C). The complete removal of the thick and impenetrable seed coat revealed a light brown to creamy endosperm covered by a whitish feathery/hairy layer in the mid portion (Figure 1D). The low germination of seeds of members of the family *Capparaceae* may be attributed to this hard seed coat and an impervious mucilaginous layer developed when exposed to water (Bahrani et al., 2008; Olmez et al., 2006). Although the hard seed coat together with the formation of a mucilaginous layer imposes dormancy, they are significant ecological adaptive features that prevent water loss and maintain seed viability during the dry season in xerophytic species including Caper (Bahrani et al., 2008; Chedraoui et al., 2017). The imbibition of water through the hilum activates metabolic activties within the endosperm resulting in seed germination (Foschi et al., 2020; Leubner-Metzger, 2003).

Effect of sulfuric acid on germination *C. erythrocarpos* seeds

Sulfuric acid, Gibberellic acid (GA_3) and mechanical scarification

Seed dormancy in many plant species is imposed by physical, morphological or physiological mechanisms that restrict seed germination and/or seedling emergence until suitable environmental conditions exist (Baskin and Baskin, 2004). The seeds of members of the family Capparaceae including C. erythrocarpos are known to exhibit both physical (seed coat imposed) and physiological (embryo imposed) dormancy to survive in arid and drought-endemic environments (Bahrani et al., 2008; Gask et al., 2008). Thus, in this study, we primed *C. erythrocarpos* seeds with sulfuric acid (H_2SO_4), either alone or in combination with GA_3 before culture on MS medium supplemented with BAP or kinetin to improve germination (Figure 1E, F).

After 5 weeks in culture on MS medium, all the seeds (100%) were swollen signifying water imbibition but no signs of germination similar to the control seeds. There was no difference in germination among seeds soaked for 3 or 6 h in varied concentrations of GA_3 (500 – 2000 mg/l). Germination in Caper starts with the cracking of the testa which requires the absorption of water by the seeds followed by embryo expansion (Foschi et al., 2023). We also observed that the inclusion of cytokinins, BAP or kinetin at a concentration of 0.5 – 2.0 mg/l in the culture medium did not induce the germination of *C. erythrocarpos*

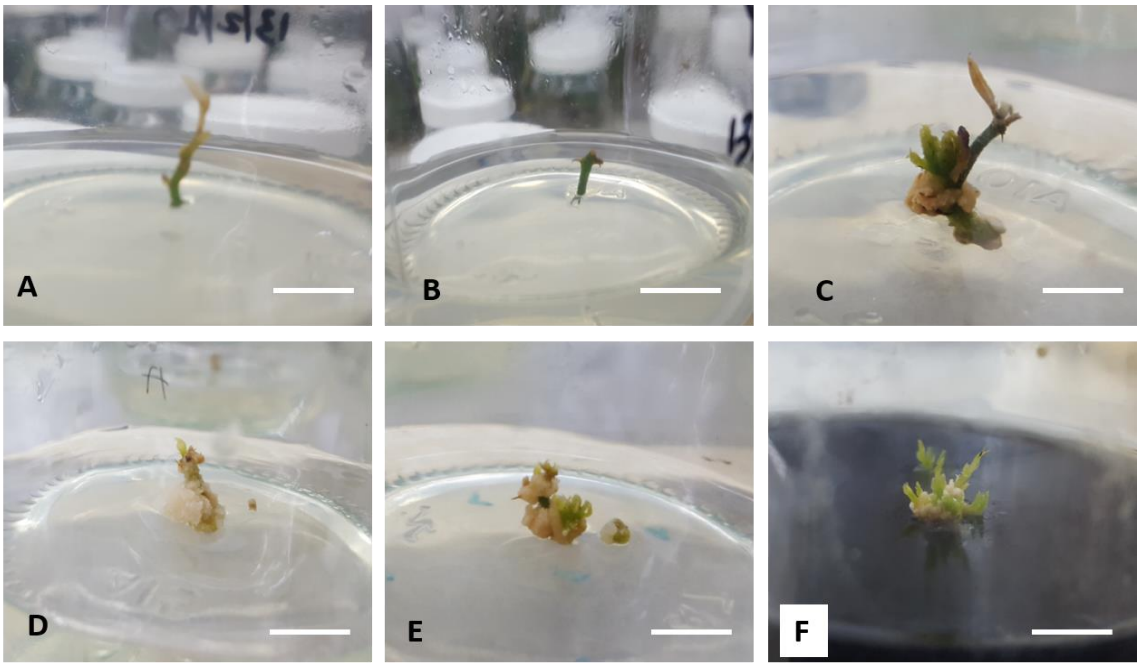

Figure 2. *In vitro* culture of *C. erythrocarpos* shoot tips and nodal cuttings. (A, B): shoot tips and nodal cuttings cultured on MS media supplemented with 0.1 mg/l each of GA_3, NAA and BAP; (C, D, E): callus formation around the base of shoot tips and nodal cuttings cultured on MS media supplemented with 0.1 mg/l each of GA_3, NAA and BAP; (F): multiple shoot formation from callus formed around the base of shoot tip explants. Bar represents 5 mm.

seeds *in vitro*. Contrarily to our report, it has been observed that mechanical scarification and pre-treatment of C. spinosa seeds with sulfuric acid and GA_3 respectively promoted germination (Awatef et al., 2017; Bahrani et al., 2008; Foschi et al., 2023). Similar results on the improvement of germination using sulfuric acid, BAP and GA_3 in C. spinosa have also been reported although the frequencies of germination varied (Ahmad et al., 2021; Al-Safadi and Elias, 2011; Germanà and Chiancone, 2009). The differences between these results and ours suggest that germination in the Capparaceae family; especially in the genus Capparis is genotype-dependent since all reports hitherto were in other Capparis species. Several authors have reported dormancy-breaking effects using a combination of growth regulators auxins, cytokinins and gibberellins (Germanà and Chiancone, 2009; Koufan et al., 2022; Wybouw and De Rybel, 2019).

The lack of germination observed after similar treatment in C. erythrocarpos in this study suggests that other factors including genotype, culture medium, endogenous plant hormones and their concentration interact to control seed germination in plants *in vitro* (Awatef et al., 2017). Additionally, the lack of germination in C. erythrocarpos seeds *in vitro* after scarification may be attributed to physiological dormancy imposed by other endogenous factors in the immature embryos which we could not determine. Physiological dormancy is known to be most common in major Angiosperm families (Finch-Savage and Leubner-Metzger, 2006). Furthermore, germination in *Capparaceae* seeds is affected by the maturity of the seeds, the fruit position, the weight and the storage period (Foschi et al., 2022; Pascual et al., 2003). In this report, we could not estimate the maturity of the seeds because they were collected from the wild. However, the deep pink colour of the fruits collected as shown in Figure 1A indicates that the fruits were ripe (Hyde et al., 2024).

Shoot tips and nodal cuttings

In vitro culture of nodal cuttings or shoot tips for the propagation of *C. erythrocarpos* may circumvent the lack of low germination of seeds in the species. The technique will also ensure large-scale clonal propagation which cannot be achieved through sexual production due to the high degree of heterozygosity of the seeds (Gask et al., 2008). Considering the potential advantages of *in vitro* propagation, we cultured shoot tips and nodal cuttings of C. erythrocarpos on MS medium for plant regeneration since they contain totipotent tissues (Figure 2A, B). After 6 weeks of culture in light, callus formation was observed at the base of the nodal cuttings and shoot tips (Figure 2C, D) which increased in size to cover the entire explant (Figure 2E) without plant regeneration.

However, further excision of the callus from the explants and transfer to fresh MS basal medium with vitamins supplemented with 2 g/l of activated charcoal and 0.1 mg/l each of GA_3, NAA and BAP induced multiple shoot

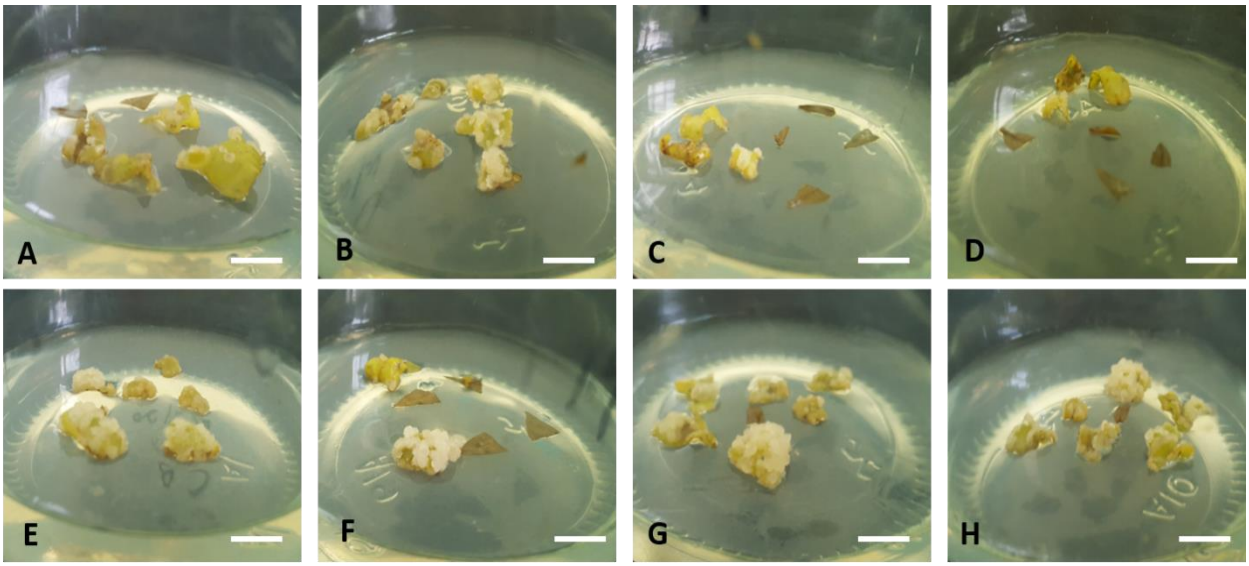

Figure 3. Callus induction in *C. erythrocarpos* leaf explants after 28 days in the dark. Callus induction in *C. erythrocarpos* leaf explants cultured on MS media supplemented with (A,B) 1 mg/l, (C,D) 2 mg/l (E, F), 3 mg/l and (G, H), and 4 mg/l 2,4-D. Bar represents 5 mm.

formation within 2 weeks of culture (Figure 2F). A total of seven shoots developed from the callus and were maintained through serial subculture every 4 weeks on the medium described above. However, after 12 weeks in culture, there was no shoot differentiation or root growth from the multiple shoots (Figure 2F). Contrastingly, there has been successful plant regeneration from nodal cuttings in C. spinosa cultured on MS media supplemented with auxins, cytokinins and meta-topolin (Chalak et al., 2003; Gianguzzi et al., 2019; Shadiadeh et al., 2012). Specifically, the auxins, 1-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA) have shown positive effects on root formation of Caper *in vitro* shoots on MS medium (Carra et al., 2012). The inability to regenerate whole plants from the multiple shoots induced in C. erythrocarpos could be influenced by genotype, media or the type of plant growth regulator (PGR), as all these factors can influence root formation (Chalak and Elbitar, 2006; Koufan et al., 2022). Thus, the investigation of different combinations of PGR and/or media for improving the growth of C. erythrocarpos shoots *in vitro* and the production of roots will enhance the rates of plant survival during the acclimatization phase (Mazri and Meziani, 2013). Ultimately, acclimatization of *in vitro* plantlets is key to ensuring the survival and field establishment of plantlets or seedlings generated for conservation or medicine.

Callus induction from young leaf explants

The culture of young leaves on MS media supplemented with 2,4-D developed callus along the cut edges similar to the nodal cuttings four weeks after culture in the dark (Figure **3**). In contrast, leaf explants cultured on MS medium without 2,4-D (control) did not develop any callus. Callus induction frequencies varied in explants cultured on the different concentrations of 2,4-D (Table 1). Callus induction was significantly higher ($p \leq 0.05$) in leaf explants cultured on MS medium supplemented with either 3 (60%) or 4 mg/l (50%) compared to explants cultured on 2 mg/l 2,4-D (17%) (Table 1). Morphologically, the calli formed were creamy in colour and mostly friable (Figure 3). In several plant species including C. spinosa, exogenous application of the auxin 2,4-D has led to successful callus induction (Elmaghrabi et al., 2017; Fahmideh et al., 2019; Kumar et al., 2014). However, the size and frequency varied depending on the concentration of 2,4-D. In the present study, the size of calli formed on MS media supplemented with lower concentrations of 2,4-D (1 or 2 mg/l) was smaller than at higher concentrations (3 or 4 mg/l). The subsequent transfer of callus to MS media supplemented with 0.1 mg/l each of BAP and NAA in light did not result in embryo or shoot formation after 2 months. Further successive transfers of calli at 2-week intervals to fresh MS media supplemented with 0.1 mg/l of BAP and NAA failed to induce somatic embryos or shoot formation in C. erythrocarpos. In contrast, successful shoot induction from the callus of C. spinosa was achieved on MS medium supplemented with 2mg/l BAP, kinetin or NAA (Elmaghrabi et al., 2017; Fahmideh et al., 2019).

Successful *in vitro* propagation in C. erythrocarpos will lead to clonal propagules production for large-scale commercial farmers interested in the cultivation of the

Table 1. Callus induction frequency in leaf explants of *C. erythrocarpos* on 2,4-D-supplemented media after four weeks.

Treatment	Total number of explants used	Number of explants that formed callus	Callus formation %
D1	30	10	33 ± 0.45^{ad}
D2	30	5	17 ± 0.22 ^{bd}
D3	30	18	60 ± 0.25 ^{ac}
D4	30	15	50 ± 0.31 ^{ac}

Percentage callus formation is calculated as a ratio of explants that formed callus/total number of explants expressed as a percentage. Percentage callus formation shows means of 5 plates per treatment ± SE on 2,4-D. Values in a column followed by different letters are significantly different from each other at $p \leq 0.05$ (Tukey's multiple comparison test).

plant for sustainable use by the herbal medicine industry. The preliminary results presented in this study highlight the possibility of developing *in vitro* plant regeneration protocols using nodal cuttings or indirect somatic embryogenesis from young leaves. However, further research aimed at improving seed germination and shoot multiplication rates using nodal cuttings, shoot tip explants or somatic embryogenesis is crucial to the scaling up of the propagation protocol. Success in this step is key for the production of sufficient quantities of plant raw materials to meet the demand for traditional herbal medicine production on a commercial scale.

Current methods of sourcing plant material from the wild are inadequate to meet the growing demands for its medicinal uses and could lead to genetic erosion or extinction. This approach presents several limitations that include the inability to determine the maturity of the seeds collected, insufficient explants (seeds or nodal cuttings) available for replicated experiments, and optimisation of propagation protocols. Caper seeds exhibit low growth potential even after imbibition mainly due to embryo-imposed dormancy (physiological dormancy) (Foschi et al., 2022). Seed maturity at harvest can influence germination percentage and our inability to estimate this parameter likely contributed to the low germination percentage recorded in this study. To overcome this limitation, the establishment of on-station plantations of *C. erythrocarpos* can provide adequate seed or explant material for scaling up the propagation protocol for commercial use. In Ghana, the unavailability of fruits or irregular fruit production observed in *C. erythrocarpos* plants found in the wild further limits the use of seed-dependent propagation methods. Therefore, the use of other parts of the plant such as nodal segments, leaves or roots for the establishment of propagation protocols could help address this limitation and achieve large-scale seedling production. For example, experiments aimed at optimizing the use of leaf explants, different PGRs and their concentration for callus induction and somatic embryogenesis would be critical in addressing the challenge of insufficient explants for scaling up of the protocol. Moreover, the optimisation of the callus induction and somatic embryogenesis protocol will facilitate the extraction of phenolic compounds and flavonoids for medicinal use.

The maintenance of clonal fidelity of *in vitro* regenerated plants is key for the medicinal use and conservation of this important medicinal plant. First, research in the area of the development of molecular techniques to complement morphological classification methods will greatly enhance the identification of the different bio/ecotypes suitable for medicine production. Upon the introduction of these bio/ecotypes into *in vitro* culture, the determination of the ploidy level of *in vitro* regenerated plants compared to conventionally propagated shoots using flow cytometry will be key to confirming genetic stability. This step is highly recommended for integration into the commercial seedling production pipeline. Ultimately, the evaluation of the bioactive compounds and composition of tissue-culture-derived *C. erythrocarpos* seedlings in comparison to seedlings sourced from the wild will be important for the herbal medicine industry to avoid adulteration of bioactive compounds. Research in this area will provide compelling evidence to promote the use of this approach for *C. erythrocarpos* and other equally important medicinal plants.

Finally, the evaluation of *in vitro* regenerated seedlings under field conditions will be critical for the herbal medicine industry and the conservation of *C. erythrocarpos.*

Conclusion

The development of an efficient *in vitro* propagation protocol to promote the commercial cultivation, improvement and conservation of *C. erythrocarpos* is possible using nodal cuttings or indirect somatic embryogenesis from young leaves. Although whole plant regeneration from seeds was not achieved in this study, the swelling of imbibed seeds suggests they are viable and justifies further optimisation of the propagation protocol. The optimization of regeneration protocols using seed, nodal and shoot tip explants will facilitate large-scale clonal propagation of *C. erythrocarpos* for medicine and conservation. The lack of existing literature on the development of *in vitro* protocols for the propagation and cultivation of this species highlights the importance of this study in addressing this gap. To the best of our knowledge,

this is the first report aiming to develop an efficient *in vitro* regeneration protocol for *C. erythrocarpos,* a species native to tropical Africa, India and the Himalayas.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This study was funded by the government of Ghana through the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission and the Centre for Plant Medicine Research (CPMR) in Mampong, Akuapem, Ghana. The authors would like to thank the Centre for Plant Medicine Research (CPMR) for providing the plant materials. We also acknowledge the support of the Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, for granting access to the Plant Tissue Culture Laboratory.

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