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Propagation of Papaya: An Overview and an Interpretation

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Abstract

Literature pertinent to the present status of papaya (*Carica papaya* L.) propagationis reviewed with the goal of providing an in-depth analysis of current research problems. Previous and recent procedures developed for propagation of papaya including sexual and asexual methods were highlighted. Particular attention has been given to tissue culture techniques. Relative problems and limitations of each technique are identified and used as pointers to the need for further research in the near future.

Key words*:* Fruit trees, Vegetative propagation, Seeds, Tissue culture, Cuttings, Papay

Introduction

Papaya, [*Carica papaya* L.], belongs to the family Caricaceae, a dicotyledonous family cultivated worldwide in tropical and subtropical America and Africa. Papaya is typically single-stemmed, erect, quick growing plant, 2-10m in height that is almost entirely propagated by seeds. Papaya plants are essentially dioecious. The plant's sex remains indiscernible until the flowering period which takes 8-12 months. The melon-like fruit of papaya is economically useful for its food value, (Cronquist, 1981), used as a popular breakfast food, in drinks and in jam, pectin and candies making. It is rich in calcium, potassium, magnesium, phosphorous and isan excellent source of pro-vitamin A and C (USDA, 2001). From the latex, which exudes from longitudinal incisions made into unripe green fruits, the enzymes chymo-papin and papin are prepared (Mezhlumyan *et al.*, 2003). These proteolytic enzymes have properties similar to gastric pepsin and are used as meat tenderizers and for medical and cosmetics industries.

Papaya is grown in Sudan in the Blue and White Nile States in small monoculture gardens, multiple-cropping systems for local consumed or traded locally due to long distances to markets and the difficulty in handling the large fruit size. It is not known exactly when and how papaya was first introduced into Sudan. It remains a vastly under researched subject of fruit trees and the value of its fruit as a source of income is underestimated as well as its nutritional uses.

Papaya is grown entirely from seedlings. Seed germination is frequently slow, transient, erratic and incomplete(Chacko and Singh, 1966). Germination inhibitors in papaya seeds play an important role during their dormancy and germination (Chow and Lin, 1991), preventing

germination of seeds while still in the fruits or prematurely after their release (Tseng, 1992). Plants grown from seeds consist of a mixture of genotypes which exhibit considerable variation in fruit size, color, shape, flavor and yield. Being essentially dioecious, propagation of papaya by seeds results in the production of large numbers of seedling males at the expense of females (Hagagy *et al.*, 1999). Commercial papaya plantations consist of dioecious plants which are overplanted and then thinned at flowering to the required ratio of male-female trees for maximum fruit set.

The purpose of this article is to present an updated review of the various methods used for the propagation of papayas with special emphasis on the application of tissue culture techniques developed for propagation of a diverse number of other fruit tree species.

Traditional Propagation Methods

Plants are propagated sexually by the use of seeds and/or asexually by the induction of adventitious organ(s) on vegetative plant parts. The application of either method depends on the plant species, the purpose of multiplication and the availability of propagation plant material.

Sexual propagation: Papaya is usually propagated by seeds. The seed in papaya is enclosed in a gelatinous sarco-testa (outer seed-coat or aril) formed from the outer integument of the ovule. Freshly harvested papaya seeds show slow, erratic and incomplete germination (Yahiro, 1979; Palanisamy and Ramamoorthy, 1987)even in seeds from which the sarcotesta has been removed (Yahiro and Oryoji, 1980) indicating that papaya seed germination is problematic. The cause (s) of low germinability of papaya seeds is still far from being understood.

Several pre-sowing treatments have been attempted to improve papaya seed germination. Removal of sarco-testa (Chow and Lin, 1991); soaking in growth regulators (Yahiro and Oryoji, 1980; Tawfik, 2002a); in potassium nitrate (Montejo *et al.*, 2002); in magnetized water (Espinosa and Fonseca-Rubio, 1997); in leaf extract and powder, (Ananthakalaiselvi and Dharmalingam, 1998); temperature extremes (Salomao and Mundim, 2000;Wood, *et al.* 2000); stratification (Yahiro, 1979); matri-conditioning (Andreoli and Khan, 1993) and *in vitro* germination (Bhattacharya and Khuspe, 2001) treatments have been conducted with only partial inhibition relief.

Cultivation of papaya is hindered by problems due to the inherent heterozygosity and dioecious nature of the crop. Papaya seedlings produced sexually through seed germination show considerable variability in commercial population and being dioecious, the desirable sex type of the produced papaya seedlings must be known to the grower before transplanting to the field to avoid the need for removal of undesirable males, thus saving time and labour. The multiplication of selected papaya trees is thus left to chance when seed produced seedlings are used. Vegetative propagation becomes imperative for the production of standard varieties from the most outstanding local types, those developed through breeding programs or those that are seedless.

Asexual propagation: vegetative propagation usually produce plants that may show greater genetic uniformity, earlier flowering and fruiting, lower fruiting height, greater fruit weight and improved yield over plants grown from seeds (Chan and Teo, 2002). Papaya is one of the fruit crops, which to date,

have defied attempts to clonally propagate by vegetative means. Like most other higher plants papaya has an indeterminate mode of growth in which the leaf axils contain subsidiary meristems, each of which is capable of growing into a shoot that is identical to the main axis. Axillary branching under natural conditions is inhibited by apical dominance. This natural growth habit of papaya plant representsa major hurdle for the vegetative propagation. Mechanical and chemicalinduction of branching has been accomplished (Sookmark and Tai, 1975; Allan and Gomes, 1995; Tawfik, 2002b), to avail planting materials for vegetative propagation. Vegetative propagation methods for some papaya coltivars have been described in the literature (Sookmark and Tai, 1975; Allan and Gomes, 1995; Ramkhelawan et *al.,* 1999; Tawfik, 2002b) but none is suitable for rapid production of large numbers of clonal transplants of desired papaya cultivars for commercial plantations. Furthermore, these techniques are often slow, time consuming, tedious, impractical, need high technical know-how, and are not widely known by growers in many papaya growing countries (Ramkhelawan *et al.,* 1999). *In vitro* propagation has become a practical means for rapid and large-scale multiplication of some fruit species (Styer and Chin, 1983). Emphasis is now placed on micro-propagation for the clonal propagation of desired papaya plants where both greater multiplication rates and plants that are disease-free can be obtained.

Tissue Culture Propagation of Papaya:

Tissue culture propagation of fruit tree species has increased in importance in recent years because it offers major production and marketing advantages over traditional propagation methods. It is often faster, promotes volume production and results in healthier plants. The feasibility of application of tissue culture propagation methods for plant propagation depends on the shoot multiplication rate from sub-cultured shoots and the percentage rooting of the shoots produced. Papaya appears to be amenable to rapid and efficient tissue culture propagation. Micro-propagation through axillary shoot proliferation (Rajeevan and Pandey, 1986; Wilna, 1988; Miller and Drew, 1990; Roque *et al.,* 2001); direct shoot formation (Agnihotri *et al.,* 2004) and adventitious regeneration of plants from callus cultures (Hossain *et al.,* 1993; Suthamathi *et al.,* 2002; Kamalakannan *et al.,* 2004) has been achieved for papaya. *In vitro* adventitious regeneration through callus permits a higher rate of shoot production with more potential for propagation than multiplication from axillary shoot proliferation but is prone to the production of off-types.

Direct shoot proliferation: Propagation of plants is achieved through direct proliferation of shoots resulting from growth and development of axillary buds in shoot tips or lateral buds or through direct induction of adventitious shoots on vegetative plants organs such as leaf or root sections. The technique of shoot tip and lateral buds culture has been widely used by plant tissue culturist for the clonal propagation of a diverse number of plant genotypes (Murashige, 1974; Karp, 1989). Shoot formation occurs via the growth and development of the pre-existing axillary buds at the base of leaves primordia of cultured shoot tips or lateral buds. The number of

plant produced is however, limited but the genetic fidelity of produced plants is ensured. Shoot tips and lateral buds of papayas have been employed by several investigators(Drew and Smith, 1986; Rajeevan and Pandey, 1986; Wilna, 1988; Miller and Drew, 1990; Rahman *et al.,* 1992; Islam *et al.,* 1993; Roque *et al.,* 2001) to clonally propagate a diverse number of desired papaya cultivars. Direct formation of adventitious shoots, through organogenesis, on cultured papaya epicotyls (Siddique *et al.,* 1999) and on young floral apices (Agnihotri *et al.,* 2004) has been, however, achieved and the genetic fidelity of such *in vitro* produced plants in other plant species has been reported (Nehra *et al.,* 1989; Jehan *et al.,* 1994). Continuous proliferating shoot culture could be established by repeatedly sub-culturing shoot tips on shoot multiplication medium after each harvest of newly formed shoots. Shoots formed can be separated, rooted, *in vitro* or *ex-vitro*, and left to grow into whole individual plants. These two clonal propagation systems have the potential to rapidly increase clones with desirable horticultural characteristics and in large numbers necessary for large-scale commercial plantations.

Indirect adventitious shoot formation: The procedure of *in vitro* propagation by the techniques of indirect shoot formation is preceded by an intermediary step of callus formation; hence the name indirect propagation. Protocols for callus formation and maintenance in papaya have been developed utilizing a variety of explants sources (Pang and Sanford, 1988; Chen and Chen, 1992; Hossain *et al.,* 1993; Mondal *et al.,* 1994; Castillo *et al.,* 1998; Pillai *et al.,* 2000; Usman *et al.,* 2002; Kamalakannan *et al.,* 2004). Production of papaya plantlets via organogenesis (Yang and Ye, 1992; Hossain *et al.,* 1993; Suthamathi *et al.,* 2002; Kamalakannan *et al.,* 2004) as well as embryogenesis (Litz and Conover, 1982; Chen *et al.,* 1987; Fitch, 1993; Zanol *et al.,* 2000) has been achieved.

Regeneration of plants from callus has often been criticized as a method of plant propagation because variation can occur. Plants regeneration through callus has been used in papaya breeding programmes and selection of new varieties with desirable horticultural characteristics in plants (Evans, 1989) and in papaya as well (Chen *et al.,* 1987; Pandey and Singh, 1988; Chen *et al.,* 1991; Khatoon and Sultana, 1994; Cai *et al.,* 1999).

Problems Associated with Tissue Culture Propagation of Papaya:

Several distinct problem areas appear to be critical to the successful development of an *in vitro* system for mass cloning of papaya. The greatest of these were:

Contamination: Successful tissue culture systems in general rest on the ability to obtain high percentage of responsive cultured tissues that are contaminants free. Contamination is a common incidence in papaya tissue culture and is a major handicap for *in vitro* culture initiation. The bud scales, nodal crevices and epidermal hairs harbor a variety of indigenous micro-flora that persist after traditional disinfestations procedures. A great deal of contamination occurred with explants collected from field grown trees (Litz and Conover, 1978; Mondal *et al.,* 1994; Saadalla and Said, 2010). Preventive measures before explant excision included the use of greenhouse-grown papaya seedlings (Schmildt and Amaral, 2002) or *in vitro* produced papaya plantlets (Rajeevan and Pandey, 1986;

Drew, 1992; Hossain *et al.,* 1993; Mondal *et al.,* 1994; Saadalla and Said, 2012), or floral apices (Agnihotri *et al.,* 2004) as sources of explant for papaya tissue culture initiation.

To minimize losses associated with contamination of papaya tissue culture systems, Roque *et al.,* (2001) and Schmildt and Amaral, (2002) immersed papaya explants in a solution of sodium hypochlorite (the commercial bleach, Clorox) at various concentrations of up to 1% and treatment duration times of up to 24 hours has been reported). Agitation in a solution of antibiotics prior to culture significantly reduced the problem of systemic bacterial and fungal contamination (Singh *et al.,* 1999; Yu *et al.,* 2001). Inclusion of antibiotics into the nutrient media (Mondal *et al.,* 1990) has been attempted to counteract contamination in papaya tissue culture with varying degrees of success.

Explant source: Proper explant selection is an important factor on which plant propagation *in vitro* depends. Various explant types have been utilized for *in vitro* culture of papayas. Shoot apices and axillary buds are most frequently used for culture initiation of many plants species (Murashige, 1974) and of papaya as well (Drew and Smith, 1986; Rahman *et al.,* 1992; Roque *et al.,* 2001). Under natural conditions, the growth and development of the axillary meristems of papaya plants is inhibited by apical dominance. Rooted cuttings of mature papaya plants have been used as a source of explants culture initiation for clonal propagation of papaya (Drew, 1988; Reuveni *et al.,* 1990).The use of young inflorescences tips by Agnihotri *et al.,* (2004), as explants, resulted in direct shoot proliferation circumventing the difficulties of working with explants obtained from mature papaya plants for *in vitro* clonal propagation of papaya.

Establishment of new *in vitro* cultures of papaya from mature woody plants is often a difficult task (Agnihotri *et al.,* 2004). The potential for morphogenic response is generally more dependent on the physiological age of the source of explants than of any difficulty in culturing. Maturation is usually associated with a declining *in vitro* regenerative capacity (Pierik, 1990). Minimum duration for bud break and high proliferation rates were associated with explants excised from younger papaya shoots than older ones (Babu *et al.,* 2002). Explants derived from mature papaya plants are more often difficult to disinfest and to establish and are slow to respond to *in vitro* culture compared to explants derived from juvenile sources (Drew and Smith, 1986; Chan and Teo, 2002; Agnihotri *et al.,* 2004). Highest establishment percentage and the greatest rate of shoot proliferation from shoot apices of 4 week-old papaya seedlings compared to lateral buds and shoot apices of mature papaya plants has been obtained by Naik and Shah, (1996). The rooting of *in vitro* produced papaya shoots derived from juvenile seedling tissue is successfully achieved (Yie and Liaw, 1977; Teo and Chan, 1994) but shoots derived from field grown papaya plants failed to root.Drew, (1988) obtained 90% rooting of shoots derived from 6-month old papaya plants compared to 30% rooting of shoots derived from mature trees. Callus formation occurs faster from zygotic embryos (Fitch and Manshardt, 1990) than from hypocotyl(Fitch, 1993) or root(Chen *et al.,* 1987) explants.

Rooting of in vitro produced shoots: successful commercial application of tissue culture techniques for papaya rests

on the ability to obtain high rates of rooting and *ex-vitro* establishment as well as the need to reproduce results at will. Rooting and establishment of *in vitro* produced papaya shoots is an erratic, sporadic and aleatory event presenting a major obstacle to large scale production of papaya clonal material. For this reason a large effort has been directed towards optimization of rooting of *in vitro* regenerated papaya shoots.

Rooting media containing reduced mineral salt concentrations (Drew, 1988; Hossain *et al.,* 1993; Dam and Le, 1997) or even agar-solidified distilled water medium devoid of mineral salts (Teo and Chan, 1994) have been employed for root formation *in vitro*. The essentiality of relatively low concentrations of indole-butyric acid (IBA) for root initiation and growth in papaya tissue culture has been established (Rajeevan and Pandey, 1986; Drew, 1987; Naik and Shah, 1996; Yu *et al.,* 2000; Roque *et al.,* 2001; Saadalla and Said, 2012). Relatively high concentrations of IBA in the rooting medium decreased root initiation and resulted in the formation of stubby, thickened roots and stunted shoots with callusing at the basal end of explants(Drew, 1988; Rajeevan and Pandey, 1986; Saadalla and Said, 2012). To overcome this problem, various media supporting agents including starch, agar, gellan gum, vermiculite were tested as substrates for *in vitro* rooting of papaya shoots. All substrates tested caused abnormal root formation with the exception of vermiculite and rock wool where normal roots were formed (Suksa *et al.,* 1998).

Prolonged exposure to IBA in culture medium was found to retard root initiation and shoot growth and exposure time of three days has been suggested by Drew, (1991) to be optimum for normal rooting. Sub-culturing on medium containing IBA negatively affect root initiation and the ability to root is completely lost by continuous sub-culturing (Litz and Conover, 1981). In contrast, Drew, (1988) initially obtained 30% rooting of shoots regenerated *in vitro* but after sub-culturing on medium containing IBA for 18 months the percentage of rooted shoots increased to 60%. The auxins, indole-acetic acid (IAA) and naphthalene acetic acid (NAA) have been less frequently used in the rooting of *in vitro* produced papaya shoots (Yie and Liaw, 1977; Hossain *et al.,* 1993; Suthamathi *et al.,* 2002).

The incorporation of activated charcoal in the rooting medium has been shown to promote(Siddiqui *et al.,* 1999) and light has been found to inhibit (Drew, 1988) root initiation of papaya shoots produced *in vitro*; hence the suggestion of covering the base of culture tubes with black paper or paint to exclude light from the lower portion of the cultures. A contradicting report by Teo and Chan, (1994) showed no effect of light on rooting percentage of papaya shoots. Drew and Miller, (1989) reported the enhancement of root initiation on papaya tissue by decreasing day length. The promotion of root initiation by temperature has been studied by Drew and Miller, (1989) where rooting can be enhanced in the range of temperature from 22ºC to 29ºC with 27ºC being optimum.

Ex-vitro rooting of papaya shoots has been attempted by Schmildt *et al.,* (1997) and by Suksa *et al.,* (1998) where *in vitro* produced papaya shoots were rooted by insertion into blocks of vermiculites or rock wool or in containers filled with vermiculite maintained in a greenhouse and

moistened with 1/2-strength MS salt solution.

Multiplication rate: the successful commercial application of tissue culture techniques for propagation of plants rest on the ability to obtain high rates of shoot multiplication as well as to reproduce the results at will. *In vitro* culture of shoot apices or lateral buds of papaya on medium containing a cytokinin often result in the proliferation of clusters of small clumps of shoots. Sequential sub-culturing of these shoot clusters on fresh medium, for further proliferation, may apparently be repeated indefinitely provided the medium is adequate for normal growth and sprouting. The rate at which precocious axillary shoots can be produced depends on the rate of leaf formation that is attainable *in vitro*. Multiplication rates vary with plant species but the range of 5- to 10-fold per 4 to 8 weeks propagation cycle is typical to plants that responded well.

In the pioneering studies with papaya low and sporadic plantlets formation was obtained (Yie and Liaw, 1977; Arora and Singh, 1978) and slow growth was experienced with initial explant excised from mature papaya plants (Drew and Smith, 1986). Increases in multiplication rate, however, occur during the first subcultures (Burikam *et al.,* 1988; Hossain *et al.,* 1991)**.** This increase in multiplication rate can be maintained through numerous subcultures (Mondal *et al.,* 1990) or diminishes with continuous subculture(Litz and Conover, 1981; Hossain *et al.,* 1991). The loss of proliferation capacity with continuous subculture on multiplication medium has been attributed to cytokinin toxicity (Drew, 1988) and to vitrification (Burikam *et al.,* 1988). Alternating re-cultures on growth regulator-free medium restored proliferation capacity (Miller and Drew, 1990; Hossain *et al.*, 1991; Naik and Shah, 1996). Accumulation of ethylene gas in the headspace of culture tubes has also been reported as a factor causing reduction in proliferation rates (Magdalita *et al.*, 1997). Facilitation of gas exchange between cultured tissues and the outside atmosphere (Lai *et al.,* 1998; McCubbin and van Staden, 2003) greatly increased shoot proliferation rates. An average multiplication rate of 10-fold with sub-culturing at 20 day interval (Rajeevan and Pandey, 1986) and a 7- fold at each subculture(Burikam *et al.*, 1988)in papaya has been reported. Miller and Drew, (1990), on the other hand, obtained 10,000 micro-cuttings from a single *in vitro* produced plantlet over a year time.

Plants regeneration through callus-mediation, may give higher rates of multiplication with more potential for mass propagation than does direct shoot proliferation from axillary buds. High efficiency callus formation and plant regeneration in papaya have been reported (Fitch, 1993; Hossain *et al.*, 1993; Castillo *et al.,* 1998; Tokumoto *et al.,* 2000). The regeneration, via embryogenesis, of more than 100 papaya plantlets per explant (Chen *et al.,* 1987), and hundreds somatic embryos (Fitch and Manshardt, 1990; Fitch, 1993; Castillo *et al.,* 1998) have been obtained. Similarly, multi-shoots were formed through indirect organogenesis via callus with more than 80 shoots per culture and a multiplication rate of 9- fold per monthly subcultures was successfully achieved by Hossain *et al.,* (1993).

The clonal status of tissue-culture derived plantlets: the clonal propagation of locally selected or known desirable papaya cultivars entails culture of shoot

apices or lateral buds obtained from mature field-grown, fully proven plants for high horticultural characteristics (Karp, 1989). Vegetative propagation of trees is easily achieved when juvenile sources of propagation materials are used. A major limitation is the need to use juvenile tissues from young trees since maturation is usually associated with a declining regenerative capacity(Babu *et al.,* 1986). Difficulties were encountered when trying to raise cultures of papaya using explants from mature trees (Rajeevan and Pandey, 1986; Agnihotri *et al.,* 2004**).** The absence of juvenile sources of explants in mature papaya trees and the difficulty of inducing juvenility in potential ones, made many investigators (Rahman *et al.,* 1992; Hossain *et al.,* 1993; Naik and Shah, 1996; Siddiqui *et al.,* 1999; Pillai *et al.,* 2000; Suthamathi *et al.,* 2002; Kamalakannan *et al.,* 2004; Saadalla and Said, 2012) to utilize explants obtained from seed or seedling maternal tissues to develop techniques and procedures appropriate for multiplication, rooting acclimation and *ex-vitro* establishment in the soil. Developed reliable and repeatable systems could possibly be applied for the direct clonal propagation from culture of explants obtained from mature papaya plants. Nonetheless, shoot apices and lateral buds obtained from mature field-grown papaya plants have been used by several investigators(Mondal *et al.,* 1990; Islam *et al.,* 1993; Chan and Teo, 2002) for culture initiation and subsequent clonal propagation of selected papaya cultivars. The genetic stability of these plants has been established after field evaluation (Pandey and Singh, 1988; Drew and Vogler, 1993; Agnihotri *et al.,* 2004).

Callus formation should be avoided if clonal fidelity is to be maintained. A wide degree of genetic variability among papaya plantlets produced via callus-mediated organo-or embryo-genesis has been documented (Arora and Singh, 1978; Litz and Conover, 1982; Hossain *et al.,* 1993). There is no convincing evidence, in spite of some unsubstantiated claims (Fitch, 1993) that callus-derived papaya plants are clonal. Production of morphologically abnormal papaya somatic embryos(Zanol *et al.,* 2000) and plantlets (Pillai *et al.,* 2000) has been reported. No extensive data is available on field performance of tissue culture callus-derived papaya plants. These plants have to be fully proven under field conditions for survival and genetic fidelity.

Incidentally, comparative studies between *in vitro* and seedling produced plants for numerous plants characteristics and fruit qualities showed that the tissue culture produced plants outperformed the seedling plants in almost all aspects studied (Pandey and Singh, 1988; Drew and Vogler, 1993)Curiously, papaya tissue culture-derived plants lose their mono-axial growth habit and produce excessive branching at the base of their trunk and flower and bear fruits close to the ground (Drew, 1988).

It could, therefore, be concluded, that the potential for the use of tissue culture techniques for propagation of papaya does exist. This review is an attempt to understand this important fruit tree, which is difficult to propagate, and suggest measures for its propagation. The examination of literature that have been reported to date give some cause for pessimisms that shoot tip culture eventually will become the preferred means of propagation; especially as the

methodology become more refined and familiar to papaya growers. Tissue culture propagation through direct shoot proliferation could offer a valuable and reliable procedure for clonal propagation of papaya. It may be possible to maintain stock plants *in vitro* for extended periods of time and to propagate these plants on a year-round basis. To date, rooting, acclimatization and hardening of tissue culture produced papaya plants remain to be an under searched micro-propagation phase. More research efforts are needed to maximize plantlet production, increase rooting percentage and to improve plantlet establishment *ex vitro* under field conditions before tissue culture techniques can be considered as an economic alternative to propagation by conventional methods.

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تكاثرالباباي(*Caricapapaya* L.): مراجعه و تفسير

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المستخلص:

تقدم هذه الورقة مراجعة حديثة و مناقشة للطرق المختلفة المستخدمة في إكثار الباباي، شجرة الفاكهه الهامه، صعبة التكاثر. تم التنوير الثر و المفصل بالمعلومات الخاصة بالتكاثر الجنسي و طرق التكاثر الخضري التقليديه و محاولات إستخدام طرق زراعة الانسجه وما صاحب ذلك من معوقات تتطلب المزيد من الدراسه و استنتجت بعض التوصيات المتعلقة بالحالة الراهنة للمعارف مع الموجهات التي يجب اخذها فى الإعتبار في البحوث المستقبلية.