IN VITRO AND *IN VIVO* PROPGATION OF PASSION FRUIT

By

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B.Sc. Agric. Sci. (Pomology), Fac. Agric., Cairo Univ., 2010

THESIS

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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: *In vitro* and *in vivo* propagation of passion fruit
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ABSTRACT

The experiments had done at nursery of pomology department, Fac. Agric., Cairo Univ. and Tissue Culture laboratory& Germplasm preservation belonging to Hort. Res. Institute, Giza; during the period of 2013 to 2015. The aim of this work was to present a suitable protocol for propagation of passion fruit (*Passiflora edulis*) through tissue culture technique by direct organogenesis, and using the seed as a classic method, also using the seedlings as explants in micro propagation of passion fruit. The results indicated that the best significant sterilization treatment was Clorox at 20% + antibiotic (Amox) -15min that gave the lowest significant contamination 33.3% and the highest significant aseptic 77.7% by using the stem nod explant. Where it gave the highest significant viability 100% and lowest dead explant zero%. MS + 100ppm/l GA3 gave 100% seed germination and the best vegetative growth that gave 9.5cm. MS supplements with 0.1mg/l BAP+ 0.2mg/l NAA +2.2mg/l kin + 1g/l Casein hydrolyzed +2g/l NH4NO3 gave the highest significant parameters of passion multiplication,viz., 100% emergence vegetative growth length (3.812cm), 5 leaf number, 2 shoot number 1.7cm shoot length and healthy plantlets . Also MS supplements with 0.2mg/l NAA (sup culture media) that gave the highest significant parameters of passion multiplication, 100% emergence, 4.66cm vegetative growth length, 5 leaf number, 3 shoot number, 0.93cm shoot length. MS with 5mg/l IBA+ 5mg/l NAA+ 1g/l Casein hydrolyzed + 2g/l NH4NO3 gave the best significant results in the number of the main roots (7root/shoot), 3cm root system length, 1cm root system diameter, and color root yellow white, leaf color was glass green

a) The seeds sterilized by 20% Clorox + Amox. Antibiotic for 15 min., where it gave 100% alive explants, 22.2% contamination and 77.22% alive aseptic explants .

b) The best significant media of seeds was MS + 100ppm/l GA3 gave 100% seed germination and the best vegetative growth that gave 9.5cm.

c) The lowest significant ½MS that gave 15% seed germination and lowest vegetative growth length 3.3cm.

The agriculture mixtures of one part of peatmose and three part of sand (1:3 volume ratio) achieved the best results of number of leave/plantlet (14), leaf area 4.3cm², root number (18), success 50%, root width 7cm, root length 10cm of vegetative growth length 55cm, shoot number 1 for the plantlets and gave 95%success, 2shoots, 38cm length of vegetative growth, 17 leave/seedy plant, 10cm² leaf area, 14 roots/seedy plant, 16cm root length and 5cm root width for the seedy plants

Key word: Passion fruit, Micropropagation, seedy propagation

DEDICATION

I dedicate this work to whom my hear felt thanks; to Allah then my Mother, my father and brothers for all the support they lovely offered along the period of my post-graduation their patience and help.

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I wish to express my thanks and pay my respect to ***Dr. MOHAMED HELMY ABD EL-ZAHER****, Professor of Pomology, Faculty of Agriculture, Cairo University, for his supervision, criticism and all his efforts and his huge contribution to the success of this work.*

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CONTENTS

INTRODUCTION

Of the estimated 500 species of *Passiflora*, in the family Passifloraceae, only one, *P.edulis* Sims, has the exclusive designation of passion fruit, without qualification. Within this species, there are two distinct forms, the standard purple, and the yellow, distinguished as *P. edulis*f. *Flavicarpa* Deg. and differing not only in color but in certain other features as will be noted further on.

The family Passifloraceae has a pan tropical distribution. Passiflora itself is absent from Africa, where many other members of the family *Passifloraceae occur* (e.g. the more pleomorphic Adenia).

Table 1. Scientific classification

Kingdom	**Plantae**
(unranked)	**Angiosperms**
(unranked)	**Eudicots**
(unranked)	**Rosids**
Order	**Malpighiales**
Family	**Passifloraceae**
Genus	***Passiflora***
Species	***P. incarnate***

Description: Passion flowers look extremely exotic, so it´s a surprise to find them growing in fields along the sides of the road. There is considerable variation between the species.

Flowers: 5 or 10 petals in a flat or reflex circle. The ivory and stamens are held atop a tall, distinctive stalk which is encircled by delicate filaments. The stigmas start out high above the anthers and slowly bend backwards to come closer for pollination. Colors include: blue, purple, pink, white and red.

Fig1. Flower of the passion fruit

The fleshy fruit: also referred to as a maypop, is an oval yellowish berry about the size of a hen egg; it is green at first, but then becomes orange as it matures. As with other passiflora, it is the larval food of a number of butterfly species, including the zebra longing and Gulf fritillary. In many cases its fruit is very popular with wildlife (Morton, 1987).

Fig2. Purple passion fruit

Medicinal use: Traditionally, the fresh or dried whole plant has been used as a herbal medicine to treat nervous anxiety and insomnia. A small clinical study suggested that in the form of a tea it may improve

the subjective quality of sleep. The dried, ground herb is frequently used in Europe by drinking a teaspoon of it in tea. A sedative chewing gum has even been produced. In cooking, the fruit of this variety

Is sometimes used for jam and jellies or as a substitute for its commercially grown South American relative *Passiflora edulis* the fruit is of comparable size and juice yield. The fruit can be eaten out of hand and historically it was a favorite of colonial settlers of the South and Native Americans alike.

Food Uses: The fruit is of easy preparation. One needs only cut it in half lengthwise and scoop out the seedy pulp with a spoon. For home use, Australians do not trouble to remove the seedlings but eat the pulp with cream and sugar or use it in fruit salads or in beverages, seedlings and all. Elsewhere it is usually squeezed through two thicknesses of cheesecloth or pressed through a strainer to remove the seedlings. Mechanical extractors are, of course, used industrially.

The resulting rich juice, which has been called a natural concentrate, can be sweetened and diluted with water or other juices (especially orange or pineapple), to make cold drinks. In South Africa, passion fruit juice is blended with milk and an alginate; in Australia the pulp is added to yogurt. After primary juice extraction, some processors employ an enzymatic process to obtain supplementary "secondary" juice from the double juice sacs surrounding each seed. The high starch content of the juice gives it exceptional viscosity. To produce a free flowing concentrate, it is desirable to remove the starch by centrifugal separation in the processing operation.

Passion fruit juice can be boiled down to a sir up which is used in making sauce, gelatin desserts, candy, ice cream, sherbet, cake icing, cake filling, meringue or chiffon pie, cold fruit soup, or in cocktails. The seeded pulp is made into jelly or is combined with pineapple or tomato in making jam. The flavor of passion fruit juice is impaired by heat preservation unless it is done by agitated or "Spin" pasteurization in the can. The frozen juice can be kept without deterioration for 1 year at 0º F (-17.78º C) and is a very appealing product. The juice can also be "vacuum-puff" dried or freeze-dried. Swiss processors have marketed a passion

Fruit-based soft drink called "Passaia" for a number of years in Western Europe. Costa Rica produces a wine sold as "Parchita Seco."

Passion fruit *(Passiflora edulis* Sims.) is an important fruit crop in many tropical countries, including Kenya. Owen (1971) reported the emergence of passion fruit growing as a viable new industry in Kenya in the early 1970's. Potential for this crop exists in Western, Nyanza and Rift Valley provinces of Kenya. Passion fruit growing is rapidly expanding to replace disease-ravaged and low value crops.

The major constraints preventing adequate production and supply of passion fruits in Kenya include poor quality seedlings, diseased planting materials, and fusarium wilt, woodiness virus, and brown spot disease, high costs of pesticides, transport, orchard establishment and maintenance. Past research priorities in Kenya focused on cultivar adaptation, trellising, pruning, fertilizer regimes,

Table 2. Nutritional value per 100 g of passion fruit fresh (3.5 oz)

Energy	**406 kJ (97 kcal)**
Carbohydrates	23.38 g
Sugars	11.2 g
Dietary fiber	10.4 g
Fat	0.7 g
Protein	2.2 g
Vitamin A	64 mg (8%)
Beta-carotene	743 mg (7%)
Riboflavin (Vit. B2)	0.13 mg (11%)
Niacin (Vit. B3)	1.5 mg (10%)
Vitamin B6	0.1 mg (8%)
Folate (Vit. B9)	14 mg (4%)
Choline	7.6 mg (2%)
Vitamin C	30 mg (36%)
Vitamin K	0.7 mg (1%)
Calcium	12 mg (1%)
Iron	1.6 mg (12%)
Magnesium	29 mg (8%)
Phosphorus	68 mg (10%)
Potassium	348 mg (7%)
Sodium	28 mg (2%)
Zinc	0.1 mg (1%)

Source: USDA Nutrients Database

Intercropping, pest control, spacing, water needs and development of brown spot-resistant varieties (Moaldm, 1997)

Nakasone and Paull (1998) reported that most commercial passion fruit producers establish plantations using seedlings, which do not spread the woodiness virus, but generate undesirable genetic variability Both yellow and purple passion fruits have been micro

propagated in many countries, but not in Kenya; micropropagation confers many advantages such as rapid production of uniform, disease-free and vigorous plants.

Passion fruits are also processed into jam, jelly, pudding, flavors for yoghurt, ice cream and other food products, or exported.

Resistant yellow passion fruit *(P. edulis* var. *flavicarpa*) rootstocks protect purple passion fruits against soil-borne diseases such as *Fusarium* wilt (*F. oxysporum* f. *passiflorae*). However, it is not yet widespread in Kenya ;so, in this trial we try to find a suitable method for propagating the passion for spreading the passion in Egyptian agriculture by offering an stuffiest number of better seedlings (Amugune *et al.*, 1993).

Gitonga (2002) decided that explants derived from such seedling if used for shoot proliferation may provide disease-free planting material. Tissue culture technology may also lead to multiplication of disease-free planting material of pest resistant cultivars derived from breeding programs, thus, leading to substantial reduction in pesticide use.

The aim of this work was to present a suitable protocol for propagation of passion fruit (*passiflora edulis*) through tissue culture technique by direct organogenesis, and using the seed as a classic method, also using the seedlings as explants in micro propagation of passion fruit.

REVIEW OF LITERATURE

1. *In vivo* seed germination

Meletti (2002) Passiflora species is almost exclusively propagated through seedlings but, there is problems related to the seed physiological quantity, unequal germination that directly damages the plant germination rates

El- Baowab (2002) revealed that GA_3 treatments (200 or 50 ppm for 24h) enhanced the germination of citrus seedlings.

Ibrahim (2003) studies the effect of gibberellic acid (500 and 1000ppm for 29hrs) on germination ability of Volkamer lemon, Sour orange, Troyer citrange and Cleopatra mandarin seedlings. The treated seed (Soaked in GA3) were sew in plastic flats (52x37x10cm) filled with sand and peatmoss 3x1 (V/V). The treatment of GA3 at 500 or 100ppm achieved 65& 71.5% germination as mean of all rootstocks; 51.73&50.80 germination rate; 7.52&7.71 leaves/ plant; 7.7&7.92cm length of seedling and 6.48&7.22cm root length, respectively.

Guzzo *et al* (2004) passiflora seedlings, collected in the wild or produced in greenhouse, were kindly furnished by Dr. Maurizio Vecchia (Ripalta Cremasca, Cremona, Italy). For each species, four to 20 seedlings were available. Mature seedlings were surface sterilized with 70% ethanol for10 min, followed by immersion in a sodium hypochlorite solution, containing 5% active chlorine for 70 min. (During the first 10 min, samples were kept under vacuum). Seedlings were extensively washed with sterile water, soaked overnight at 35°C, and then for 24 h at room temperature. Germinated plantlets were transferred into Magenta vessels and grown in vitro in hormone-free