A functional decision support system for management of Graphiola leaf spot (*Graphiola phoenicis*) disease during hardening of date palm tissue cultured plants in a greenhouse

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Summary

A functional decision support system (DSS) was developed through optimising values of parameters favour faster growth and development of tissue cultured plantlets of date palm and suppression of disease infection. The programming of control devices was further managed based on the ambient environmental conditions, which required variable simulation periods and a plan for controlling of all systems for maintaining the greenhouse environment. During the process of plant hardening, the plants were infected by Graphiola leaf spot (*Graphiola phoenicis*). The moderate temperature, and high humidity inside the greenhouse, accompanied by short days and frequent occurrence of fog during winter months, favoured the development of smut infection. It is required to manage an extended photoperiod for 16 hours with 15000 lux light intensity provided by white fluorescent tube lights and incandescent bulbs inside the greenhouse. The integrated practices of enhanced photoperiod and light intensity under the greenhouse in combination with fungicides application to plants were found very effective in suppressing the intensity of the disease and its adverse effects on the plants. Using this decision support system (DSS), the tissue cultured date palm plants cv. Barhee has been successfully hardened and managed disease and transplanted in the field for further studying establishment, survival, plant growth and fruiting-related parameters.

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Introduction

Date palm (*Phoenix dactylifera* L.) belongs to the family Arecaceae and is a perennial, monocotyledonous and dioecious tree. It has immense socioeconomic, environmental and ecological values, particularly in the arid and semi-arid regions of the world (Kriaa et al. 2012). The plants can tolerate extremes of temperature and saline conditions from arid areas. It also has several nutritional and health benefits that have triggered a lot of biotechnological interventions related to its micropropagation, improvement and preservation of elite germplasm. The availability of quality planting material is a significant limiting constraint in its area expansion programme. So far, it is multiplied by suckers which is cumbersome and not an efficient method for its multiplication. Some organizations have tried to develop *in vitro* protocol, but it is varietal specific, and demands for planting materials of commercial varieties are enormous. The Indian government is also supporting date palm growers to import the tissue culture planting very expensive material. Therefore, the alternative is our protocol for mass multiplication commercial date palm varieties (Kumar et al. 2020). Micropropagation of date palm through somatic embryogenesis and hardening of plantlets are lengthy processes which require frequent sub-culturing of several steps and careful attention. Otherwise, the casualty rate will be very high. Fifty to ninety per cent loss has been reported in date palm plantlets during acclimatization. To overcome the propagation problems in date palm and mass multiplication of elite genotype *in vitro,* micropropagation is a successful technique which provides rapid production of genetically uniform, true-to-type, good quality and disease-free planting materials of date palm around the year (Bhattacharjee, 2006; Haldhar & Maheshwari 2021; Kumar et al. 2020).

The ultimate success of the tissue culture technique depends on proper hardening and survival of plants with faster growth and development. We have established a three-step plant hardening facility with three compartments, greenhouse environmental control devices, misting/fogging, and micro irrigation systems. The sensors, timers and speed regulators controlled important parameters such as temperature, humidity, airflow, light intensity, photoperiodic control, and watering. A functional decision support system (DSS) was developed through optimising values of parameters favour faster growth and development of tissue cultured plantlets of date palm and suppression of disease infection. Control device programming was further

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managed based on the ambient environmental conditions, which required variable simulation periods plan for controlling all systems for maintaining the greenhouse environment. The moderate temperature, high humidity inside the greenhouse accompanied by short days and frequent fog during winter months favoured the development of smut infection, *i.e* Graphiola leaf spot. This disease is caused by the fungal pathogen *Graphiola phoenicis*. It is a unique fungus in appearance and life cycle, but it is widely distributed throughout the date palm-growing world. While numerous palm species have been identified as hosts of this fungus, the disease is most prevalent in Florida on *Phoenix* species, such as *Phoenix canariensis* (Canary Island date palm) and *Phoenix dactylifera* (date palm). It is rarely observed on *Phoenix sylvestris* (wild date palm). Graphiola leaf spot is caused by *Graphiola phoenicis* (Moug) Poit., which is a smut fungus. It develops sub-epidermally, in small areas on both sides of the pinnae leaves, on the rachis and the leaf base. The numerous fruiting structures emerge as small yellow/brown to black sori, 1 to 3 mm in diameter, with two layers. These sori are abundant on three year-old leaves, conspicuous on two year-old, but absent or infrequent on one year-old leaves. This is because of this pathogen's 10 - 11 month incubation cycle. On a leaf, sori are abundant on the apical pinnae, less productive on the middle section, becoming even less on the basal area. The average 6- 8 year life of date palm fronds will be reduced to 3 years by *Graphiola* disease, and heavily infected leaves die prematurely, consequently decreasing the palm yield (Haldhar et al. 2017; Guar 2000).

Graphiola leaf spot disease is most common in Egypt (Delta region and Fayum) but absent in the less humid oases. In Saudi Arabia, Libya (Edongali 1996) and, Kenya (Kung'u & Boa 1997), Qatar and Yemen (Sattar et al. 2013), but absent in Iraq. Reports of this disease also originate from Algeria and the USA. Around the world, it is the most widely spread disease and occurs wherever the date palm is cultivated under humid conditions - mostly marginal date growing areas (Mediterranean coast) but also in the southernmost humid regions of Mali, Mauritania, Niger and Senegal. For field-grown palm the control measures Lodha (2003) include leaf pruning coupled with treatment with Bordeaux mixture or any large spectrum fungicide (mancozeb, cupric hydroxide, cupric hydroxide + maneb, or copper oxychloride + maneb + zineb; 3 to 4 applications on a 15-day schedule after, sporulation, have been recommended). Nixon (1957), Singh et al (1970) and Haldhar et al. 2022 reported genetic tolerance in some varieties (Barhee, Adbad, Rahman, Gizaz, Iteema, Khastawy, Jouzi and Tadala) under field condition. However, till now no reports are available on the occurrence of smut problem in tissue culture plants of date palm during hardening and its effective control measures.

Therefore, this is the first study in this line for managing smut problem during hardening process in the green house. The integrated practices of enhanced photoperiod and light intensity under green house in combination with fungicides application to plants were found very effective to suppress the intensity of disease and its adverse effects on the plants. Using this decision support system (DSS), the tissue cultured date palm plants cv. Barhee has been successfully hardened and transplanted in the field for the establishment, survival, growth, and fruiting related parameters.

Materials and Methods

One hundred sixty plants of date palm cultivar 'Barhee' were procured from Anand Agricultural University, Anand (Gujarat), India at the secondary hardening stage under the collaborative project on date palm tissue culture and field demonstration. The are plants kept for further hardening, growth and development under greenhouse unit. This greenhouse unit is composed of a step plant hardening facility fitted with environmentally controlled micro-processed based devices with misting/fogging and micro irrigation systems etc. Important parameters such as temperature humidity, air flow, light intensity, photoperiodic control, and watering were controlled through sensors, timers and speed regulators. Plant hardening temperature was kept at 12±2°C at night, 35±2oC during the day, and 40-60 % relative humidity. During January, the plants were infected by Graphiola leaf spot (Graphiola phoenicis) with the symptoms of small holes on both sides of leaves and yellow spore masses underneath leaves (Fig. 1&2). For its management, the leaves of infected plants were pruned out, and plants were sprayed weekly with copper oxychloride 1.0 gm/ l and bavastin 1.0g/l fungicides alone or in combination with extended photoperiod for 16 hours with 15000 lux light intensity provided by white fluorescent tube lights and incandescent light bulbs. A total six sprays of fungicides were applied to all plants under the experiment for disease management.

A functional decision support system (DSS) was developed through optimising values of parameters that favoured faster growth and development of tissue-cultured plantlets of date palm and suppression of disease infection. Control device programming was further managed based on the ambient environmental conditions, which required variable simulation periods plan for controlling all systems for maintaining the greenhouse environment. This facility was used for hardening, faster growth and development of the date palm plantlets and minimization of smut disease during the hardening process.

Result and Discussion

Variation in the incidence of Graphiola leaf spot was observed with all treatments alone or in combination (Table 1). The study shows that negligible infection was recorded with combined treatments of Copper oxychloride + Bavastin + Extended photoperiod for 16 hrs, and consequently, fewer sori on the leaf surface were noticed. The symptoms (sori) exhibited a drastic reduction of the leaf area covered by the fungus. This is confirmed by Singh et al. (1970), by noticing a reduction in leaf area and a decline in chlorophyll level in the leaves due to infection by *Graphiola phoenicis*. A comparison of leaf surface revealed that lower surface of leaf trapped more number of sporidia than the upper surface (Table 1) and therefore, the number of sori was higher and showed significant variation among all treatments. Similarly, the distribution of sori in terms of leaf position was more pronounced to older leaves reported by Lodha (2003). Temperature ranging from 28-36°C in summer and 10-27°C in winter under greenhouse accompanied by water condensation in the night and early morning hours, particularly during winter months were favourable for continue development of infection. According to Sattar et al. (2013), temperature ranging from 32-38°C in summer and 18-27°C in winter, accompanied by heavy dew in the night and early morning hour's regardless of duration are favourable for subsequent development of smut infection in date palm.

The improvement of plant health under the integrated system by extended photoperiod over the treatments along with chemical spray of copper oxychloride and bavastin may be due to enhanced photosynthesis by light under short days and fog conditions prevailing in arid regions during winter months. Further, these results are in close conformity with the findings of Singh et al. (1970) who reported negative effect of smut on chlorophyll control.

Table 1. Effect of different treatments on smut management: Disease incidence and number of sori on both surfaces of tissue cultured date palm plant leaves

Treatment	Treatment detail	Disease incidence (%)	Number of sori on leaves	
			Upper surface of leaf	Lower surface of leaf
T_1	Copper oxychloride	33.33 (35.25)	32.33	42.67
T_2	Bavastin	33.33 (35.25)	41.00	62.33
T_3	Copper oxychloride + Bavastin	18.33 (25.31)	12.67	20.67
T_4	Copper oxychloride + Extended photoperiod for 16 hrs	20.00 (26.57)	0.00	8.00
T_5	Bavastin + Extended photoperiod for 16 hrs	13.33 (21.34)	0.00	7.33
T_6	Copper oxychloride + Bavastin + Extended photoperiod for 16 hrs	8.33 (16.60)	0.00	3.67
	SEm±	1.23	1.15	24.11
	CD5%	3.81	3.53	1.07
	CD1%	5.33	4.95	3.30

Furthermore, combined application (Table 2) of copper oxychloride and bavastin with extended photoperiod for 16 hrs significantly improved the overall plant growth characteristics of tissue cultured date palm plants during secondary hardening in green house. Plant height (26.00 cm), number of leaves/plant (7.00), length (24.00 cm) and width (3.27 cm) of leaves were recorded maximum in treatment T_6 followed by T_5 and minimum in treatment T_2 (only bavastin) for these plant growth parameters. Combined application of copper oxychloride + bavastin (T_3) or a single application of either of these fungicides along with extended photoperiod (T_4 or T_5) were performed better than only one fungicidal (T_1 or T_2) application concerning growth characteristics (Table 2) and management of smut (Table 1) in tissue cultured date palm plants. This integrated decision support system was more effective than a single application of repeated pesticides (copper oxychloride/ bavastin) in the greenhouse in managing Graphiola leaf spot and lowering pesticide sprays by reducing disease incidence and faster growth of plants during plant hardening under the greenhouse.

The results obtained in this study are corroborated with the findings of Nestel et al. (2019) in the management of the Ethiopian fruit fly in greenhouse crops. They reported that the decision support system followed by greenhouses received less pesticide (67%) application than control greenhouses in managing this insect in melons. Marimon et al. (2020) developed a DSS based on degree days for controlling peach powdery mildew in Spain, and they concluded that 220 - accumulated degree days alert programme was found effective in reduction (33%) of fungicide applications by statically relevant minimizing disease incidence in peach fruits. Cohen et al. (2008) successfully managed the Mediterranean fruit fly in citrus orchards in Israel by integrating the decision support system IPM strategy. DSS helped reduce pesticide utilization to control fruit fly in citrus orchards and agreed on the acceptance of farmers and pest inspectors to adopt the system.

Table 2. Effect of different treatments on smut management and growth and development of tissue cultured date palm plants

Treatment	Treatment detail	Number of leaves per plant	Width of leaves(cm)	Length of leaves (cm)	Plant height (cm)
T_1	Copper oxychloride	5.33	2.13	20.00	21.33
T_2	Bavastin	4.33	1.90	16.33	17.33
T_3	Copper oxychloride + Bavastin	5.33	3.13	20.67	23.33

T_4	Copper oxychloride + Extended photoperiod for 16 hrs	5.67	3.07	21.33	24.00
T_5	Bavastin + Extended photoperiod for 16 hrs	6.67	3.17	23.00	24.33
T_6	Copper oxychloride + Bavastin + Extended photoperiod for 16 hrs	7.00	3.27	24.00	26.00
	SEm±	0.38	0.26	0.62	0.43
	CD5%	1.19	0.81	1.92	1.33
	CD1%	1.66	1.13	2.69	1.86

Figure 1. Tissue cultured plants of date palm cv. Barhee under green house

Figure 2. Graphiola leaf spot (*Graphiola phoenicis*) infected plant and fruiting bodies of the false smut fungus on the upper side of the leaf

Declaration of Interests

The authors have no conflict of interest to declare.

Data Sharing

All relevant data are within the manuscript.

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