



Integrated management of *Graphiola* leaf spot (*Graphiola phoenicis*) in tissue cultured date palm saplings during plant hardening stage

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Abstract

Tissue culture plants of date palm cultivar Barhee produced through somatic embryogenesis at AAU, Anand were primarily hardened and supplied to ICAR- CIAH for secondary hardening and field evaluation. The plants were kept under greenhouse for secondary hardening and further growth and development of plants for achieving transplantable size of plants for field plantation. During the process of plant hardening, the plants were infected by *Graphiola* leaf spot (*Graphiola phoenicis*) with the symptoms of small spots on both sides of leaves and yellow spore masses on underneath of leaves. The moderate temperature, high humidity inside the green house accompanied by short day and frequently occurrence of fog during winter month favored the development of smut infection. For its management, the leaves of infected plants were pruned out and plants were sprayed weekly with copper oxychloride and bavastin fungicides alternatively. 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 these practices, the plants have been successfully hardened and planted in the field.

Key words: Date palm, graphiola, tissue culture plants.

Introduction

Micropropagation of date palm through somatic embryogenesis and hardening of plantlets are lengthy processes, which require frequent subculturing of several steps and careful attention. Otherwise, casualty rate will be very high. Fifty to ninety percent loss has been reported in date palm plantlets during acclimatization. This disease is caused by the fungal pathogen, *Graphiola phoenicis*. It is a unique fungus, both 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 *G. phoenicis* (Moug) Poit., which is a smut fungus. It develops sub-epidermal, in small spots on both sides of the pinnae leaves, on the rachis and on 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 the 10 - 11 month incubation cycle for this pathogen. On a leaf, sori are abundant

on apical pinnae, less abundant on the middle section becoming even less on the basal section. The normal 6- 8 year life of date palm fronds will be reduced to 3 years by *Graphiola* disease and heavily infected leaves die prematurely which consequently reduce yield of the palm (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 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 southern most 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) and Singh et.al.(1970) reported genetic tolerance in some varieties (Barhee, Adbad, Rahman, Gizaz, Iteema, Khastawy, Jouzi and Tadala) under field condition.

However, no reports are available on the occurrence of smut problem in tissue culture plants of date palm during hardening and its effective control measures.

Materials and Method

Under collaborative project on date palm tissue culture and field demonstration, one hundred sixty plants of Barhee variety were procured from Anand Agricultural University, Anand, Gujarat at secondary hardening stage during the month of November 2015. The plants kept for further hardening, growth and development under greenhouse having temperature $12\pm 2^{\circ}\text{C}$ during night and $35\pm 2^{\circ}\text{C}$ during day time and 40-60 % RH. During the month of January the plants were infected by *Graphiola* leaf spot (*Graphiola phoenicis*) with the symptoms of small spots on both sides of leaves and yellow spore masses on underneath of leaves. 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 hrs with 15000 lux light intensity provided by white fluorescent tube lights and incandescent light bulbs. Total 06 spray of fungicides were applied to all plants under experiment.

Result and Discussion

Variation in the incidence of *Graphiola* leaf spot was clearly observed with all treatments alone or in combination (Table 1). It appears from the study that negligible infection was recorded with combined treatments of Copper oxychloride + Bavastin + Extended photoperiod for 16 hrs and

consequently less number of sori on the leaf surface was 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 in reduction in leaf area and decline in chlorophyll level in the leaves due to infection by *Graphiola phoenicis*. 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 $28-36^{\circ}\text{C}$ in summer and $10-27^{\circ}\text{C}$ in winter under green house accompanied by water condensation in the night and early morning hours particularly during winter months were favorable for continue development of infection. According to Sattar et.al.(2013) temperature ranging from $32-38^{\circ}\text{C}$ in summer and $18-27^{\circ}\text{C}$ in winter accompanied by heavy dew in the night and early morning hours regardless of duration are favorable for subsequent development of smut infection in date palm.

The improvement of plant health under integrated system by extended photo period over the treatments alone with chemical spray of copper oxychloride and bavostin may be due to enhanced photosynthesis by light under short day and fog conditions prevail in arid region during winter months. Further these results are in close conformity of the findings of Singh et.al.(1970) as 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 surface of date palm leaves

Treatment	Disease incidence (%)	Number of sori on leaves	
		Upper surface of leaf	Lower surface of leaf
Copper oxychloride	33.33 (35.25)	32.33	42.67
Bavastin	33.33 (35.25)	41.00	62.33
Copper oxychloride + Bavastin	18.33 (25.31)	12.67	20.67
Copper oxychloride+Extended photoperiod for 16 hrs	20.00 (26.57)	0.00	8.00
Bavastin +Extended photoperiod for 16 hrs	13.33 (21.34)	0.00	7.33
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

Table 2. Effect of different treatments on smut management, saplings growth and development

Treatment	Number of leaves per plant	Width of leaves(cm)	Length of leaves (cm)	Plant height
Copper oxychloride	5.33	2.13	20.00	21.33
Bavastin	4.33	1.90	16.33	17.33
Copper oxychloride + Bavastin	5.33	3.13	20.67	23.33
Copper oxychloride + Extended photoperiod for 16 hrs	5.67	3.07	21.33	24.00
Bavastin +Extended photoperiod for 16 hrs	6.67	3.17	23.00	24.33
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



Fig. 1: Healthy tissue cultured plants of Barhee variety



Fig. 2: Graphiola leaf spot (*Graphiola phoenicis*) infected plant. Fruiting bodies of the false smut fungus, on the upper side of the leaf

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