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Original Article

Detection of adaptive genetic diversity and chemical composition in date palm cultivars and their implications in controlling red palm weevil, *Rhynchophorus ferrugineus* Oliver

Detecção da diversidade genética adaptativa e composição química em cultivares de tamareira e implicações no controle do bicudo vermelho da palmeira, *Rhynchophorus ferrugineus* Oliver

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Abstract

This study, about RPW and date palms, is under the scope of date palm bioecology and nutrition (nutritional ecology) which includes the integration of several areas of research such as date palm biochemistry, genetics, and RPW infestation behavior through various date palm cultivars. Date palm (*Phoenix dactylifera* L.; Arecaceae) production is under threat from the red palm weevil (RPW), *Rhynchophorus ferrugineus* Oliver. A better understanding of genetic diversity within date palm cultivars can be useful for its implementation within the insect IPM program in the future. Three indices, namely simple-sequence repeats (SSR) markers to elucidate genetic diversity, chemical components, and a natural infestation index of RPW, were used to evaluate the resistant or susceptible date palm cultivars in Qassim. Based on a field survey of RPW infestation within 79 date palm farms involving 11 cultivars at Qassim, the sensitivity and resistance cultivars were determined. The resistant date palm cultivars were Nabtat Ali, Shakrah, red Sukary, and um Kobar which had the lowest degree of RPW abundance %. Values of the essential minerals, nitrogen, phosphorus, potassium, and calcium within the date palm cultivars were also estimated. RPW abundance % was negatively correlated with the calcium content of date palm cultivars. The principal component analysis (PCA) revealed that the calcium content and RPW abundance % were highly affected by the cultivars. SSR markers of the date palm cluster tree divided genotypes into two main groups at similarity coefficients between 0.56 and 0.91. The 1st group included; Nabtet Ali, Red Sukary, Um Kobar, and Shakrah with similarity coefficients between 0.56, this group was the most resistant cultivars. Therefore, SSR markers were able to characterize and resolve genetic diversity in date palm cultivars for RPW resistance. When SSR markers coupled with higher calcium (Ca) content can efficiently replace indices in characterizing resistant date-palm genotypes with a high confidence level. Integration between date palm genetic diversity, chemical structures, and RPW infestations rates promoted the understanding of the interplay between the diversity of RPW management (short-time scale), and the resistance genes, plant nutrition, and dynamics of the diversity of RPW through domestication and diversification (long-timescale). Therefore, our results may lead to a change in RPW control strategies by switching to using safe alternative pesticide control methods (Resistant cultivars of date palm), which are underestimated and may reveal the impact of low-cost, but highly effective agricultural practices in the field of date production in the world. Understanding the genetic structure and calcium content of date palm cultivars mechanisms could help to predict date palm resistance against RPW populations in the new IPM strategy in RPW control.

Keywords: date palm, *Phoenix dactylifera*, date palm resistant cultivars, genetic analysis, *Rhynchophorus ferrugineus*, DNA sequence, genetic variation, plant gene resistance, date palm chemical analysis, major nutritional elements.

Resumo

Este estudo, sobre RPW e tamareiras, está no âmbito da bioecologia e nutrição da tamareira (ecologia nutricional) que inclui a integração de várias áreas de pesquisa, como bioquímica da tamareira, genética e comportamento de

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infestação de RPW através de vários cultivares de tamareira. A produção da tamareira (*Phoenix dactylifera L.*; *Arecaceae*) está ameaçada pelo gorgulho vermelho da palmeira (RPW), *Rhynchophorus ferrugineus Oliver*. A compreensão mais aprofundada da diversidade genética dentro dos cultivares de tamareiras pode ser útil para sua implementação no futuro programa de MIP de insetos. Três índices, ou seja, marcadores de sequência simples (SSR) para elucidar a diversidade genética, componentes químicos e um índice de infestação natural de RPW, foram utilizados para avaliar as cultivares de tamareiras resistentes ou suscetíveis em Qassim. Com base em uma pesquisa de campo da infestação de RPW em 79 fazendas de tamareiras envolvendo 11 cultivares em Qassim, as cultivares de sensibilidade e resistência foram determinadas. As cultivares de tamareiras resistentes foram Nabtat Ali, Shakrah, red Sukary e um Kobar, que apresentaram o menor grau de abundância de RPW. Também foram estimados os valores dos minerais essenciais, nitrogênio, fósforo, potássio e cálcio nas cultivares de tamareira. A porcentagem de abundância de RPW correlacionou-se negativamente com o teor de cálcio das cultivares de tamareira. A análise de componentes principais (PCA) revelou que o teor de cálcio e a abundância de RPW % foram altamente afetados pelas cultivares. Marcadores SSR da tamareira dividiram os genótipos em dois grupos principais com coeficientes de similaridade entre 0,56 e 0,91. O 1º grupo incluiu; Nabtet Ali, Red Sukary, Um Kobar e Shakrah com coeficientes de similaridade entre 0,56, este grupo foi o de cultivares mais resistentes. Portanto, os marcadores SSR foram capazes de caracterizar e resolver a diversidade genética em cultivares de tamareiras para resistência a RPW. Quando os marcadores SSR associados ao maior teor de cálcio (Ca) podem substituir com eficiência os índices na caracterização de genótipos de tamareiras resistentes com alto nível de confiança. A integração entre diversidade genética da tamareira, estruturas químicas e taxas de infestação de RPW promoveu a compreensão da interação entre a diversidade de manejo de RPW (escala de tempo curto) e os genes de resistência, nutrição de plantas e dinâmica da diversidade de RPW por meio da domesticação e diversificação (longo prazo). Portanto, nossos resultados podem levar a uma mudança nas estratégias de controle de RPW, passando a usar métodos alternativos seguros de controle de pesticidas (cultivares resistentes de tamareira), sendo subestimados e podem revelar o impacto de práticas agrícolas de baixo custo, mas altamente eficazes no campo de produção de tâmaras no mundo. Compreender a estrutura genética e o teor de cálcio dos mecanismos dos cultivares de tamareira pode ajudar a prever a resistência da tamareira contra populações de RPW na nova estratégia de IPM no controle de RPW.

Palavras-chave: tamareira, *Phoenix dactylifera*, cultivares resistentes à tamareira, resistência de plantas, *Rhynchophorus ferrugineus*, sequência de DNA, variação genética, resistência gênica da tamareira, análise química da tamareira, principais elementos nutricionais.

1. Introduction

Date palm (*Phoenix dactylifera* L.; Arecaceae) is one of the oldest fruit crops grown in the arid and semi-arid regions and is currently cultivated in nearly 30 countries on the Asian, African, American, and Australasian continents (Chao and Krueger, 2007; Krueger, 2021). There are over 100 million date palms worldwide, of which 60% are in the Middle East and North Africa (MENA) (El Bouhssini, 2018). Dates are one of the great income crops and form a major food source for local populations in the Middle East and North Africa. Therefore, it plays a significant role in the economy, society, and environment in the previously mentioned areas (Al-Abbad et al., 2011). Dates fruits, as a food, provide nutritional security for millions of humans in the arid regions of the world, and also have a significant role in nutrition, due to their high content of essential nutrients (Chao and Krueger, 2007). Globally, Date's production has increased from 1.8 million tons in 1962 to over 8.0 million tons at present (El Bouhssini, 2018).

Botanically, date palm plants are considered diploid, perennial, dioecious, monocotyledonous, and adapted to the arid and semi-arid agroecosystem (Vayalil, 2012). Global date palm fruit production increases with the increasing human population, especially in the Middle East, despite current and future challenges (Chao and Krueger, 2007). Thousands of date palm cultivars exist in different date-growing countries and may be associated with genetic and morphological differences (Vayalil, 2012). Different genetic marker systems have been used to study genetic relationships among date palm cultivars (Jaradat, 2015; Mathew et al., 2015). Date palm cultivation involves a mix of clonal and sexual propagation and each female date palm named type (for instance Medjool or Khalas) is a cultivar, that is a clone, multiplied only through vegetative. Mostly, date palm farmers benefit from asexual reproduction through offshoot multiplication, while sexual reproduction only leads in rare cases to progenies having equivalent or superior fruit qualities (Hadrami et al., 2011). The long life cycle, long period of juvenility, and dioecism of date palms lead to big challenges in palm breeding (Hadrami et al., 2011). Concerning Gulf countries, date palms have primary importance, more generally, as the keystone of this arid and semi-arid ecological system. As well as, It is considered the cornerstone of the local economy in exportation to large global trade centers (Rathore et al., 2020).

At least 132 species of mite and insect pests are associated with date palm trees belonging to 10 insect orders and 42 different families. Of these species, the red palm weevil (RPW), *Rhynchophorous ferrugineus* Olivier (Coleoptera: Curculionidae), is identified as one of the most major invasive key and destructive date palm pests which attacks over 40 palm cultivars, worldwide (El-Shafie et al., 2017). *R. ferrugineus* is a kind of weevil belonging to the Curculionidae family, which is native to Southeast Asia. This RPW is considered to be a pest to Palmaceae or Palm Family, particularly date palms, which has caused considerable economic damage to the date palm industry worldwide. Over the last four decades, *R. ferrugineus* has been spreading rapidly across the Middle East, into Europe, Central America, and the Caribbean to cause a global food chain crisis, and nowadays, it is present in more than 60 countries (Witt et al., 2020). *R. ferrugineus*, is seen as one of the most destructive pest-threatening date palm production and associated industries (Dembilio and Jaques, 2015; Al-Dosary et al., 2016; Knutelski et al., 2021; Abdel-Baky et al., 2021; Abdel-Baky et al., 2022, 2023).

Date palm diversity is shaped by biological and social processes that interact at different spatiotemporal scales. The domestication of many economically valuable crops such as palms and their selective breeding methodology altered the plant defense mechanisms, which affected the interactions between RPW and their host (Pintaud et al., 2011; Turcotte et al., 2014; Chen et al., 2015; Agut et al., 2018). The continuance of date palm domestication led to the emergence of new cultivars that had specific genetic, chemical, and morphological characteristics. Date-palm plants reproduce basically in two ways, i) sexually by seeding and/or ii) asexually by tissue cultures (Moussouni et al., 2017).

Date palm domestication is the result of extending cultivation and artificial gene selection, which lead to genetically based adaptations in favor of human consumption and grow-up in arid and semi-arid agricultural conditions. Many date palm cultivars are often associated with “domestication syndrome” which are exceptions that mostly apply to typical crop hosts such as date palms. Although some recurrent date-palm breeding changes were identified, the results confirmed diversity among the palm cultivars through the domestication process and vary according to the age of date palm domestication and plant growth stages. Many date palm cultivars are often associated with “domestication syndrome” which are exceptions that mostly apply to typical crop hosts such as date palms. Using this approach, the longest-lived and best-reproduced evolutionary experiences in human history were exploited that may affect date palm resistance against RPW and others. Accordingly, there is no doubt that all the problems facing date and/or ornamental palm cultivation worldwide are the result of the spread and adaptation of RPW to the surrounding environment (El-Shafie, 2014; Wang et al., 2015).

Hosts from the wild or cultivated plants may have a defense system to protect themselves from different pests attacking through the presence of some morphological characters and or/ biochemical components, as well as to be modified genetically that may hamper harmful insects from feeding or laying their eggs (Rani and Jyothsna, 2010). Because of the integrated pest management (IPM) strategy, host-plant resistance requires the premeditated use of the resistant crop cultivars, either alone or in combination with other IPM components, to reduce the harmful insect impacts on date palm yield or quality (Sharma and Ortiz, 2002; Jindal et al., 2013). Plant resistance to insect pests is a plant expression of some resistance-related characteristic that overlaps with one or more aspects of the interaction between the insect pest and a target plant (Stout, 2013, 2014). The traditional approach of developing and using any resistant plant species in IPM programs maybe pass by four steps: 1- Screening (an assessment of plant germplasm to obtain a resistant genotype), 2- Classification (which is the assignment of resistance phenotypes to classes of antibiotics, antixenosis, tolerance), 3- Breeding (which is the introducing responsible resistance genes into acceptable plant hosts from an agricultural point of view), 4- Implementation (which means incorporating resistant cultivars into management programs) (Stout, 2014). Therefore, using resistant plants is considered one of the key IPM components that may affect the population density of an insect pest, damage, beneficial organisms’ efficiency, and dose concentrations of insecticides in agricultural ecosystems (Fathipour and Sedaratian, 2013). As well, Characters of host plant resistance are one of the key important components of integrated pest management programs which is a safe alternative control method (Brzozowski and Mazourek, 2020).

Considering the identification of resistance genes and certain biochemical elements in date palms and their correlation with RPW infestation, the information in this regard is scarce, To the best of our knowledge, this study considers the first report concerning screening the resistance genes in certain date palm cultivars and links those resistance cultivars and certain biochemical components with RPW infestation rates. Date palm trees’ characteristics may influence their interaction with RPW, which is being considered to develop new ideas for RPW control strategies in the future. Therefore, it was necessary to use more modern methods to determine some genetic characteristics and chemical components (major nutritional elements) that distinguish some Qassim date palm cultivars from others in Saudi Arabia, which may be useful in being an unsuitable host for the spread of the red palm weevil. These characteristics can be transferred to the new cultivar that lacks them through date palm propagation by tissue culture techniques. Consequently, this study aimed to detect the genetic diversity of date palm cultivars, as well as, their infestation rates, which could provide IPM decision-makers with sufficient information to enhance the effectiveness of RPW control. Therefore, the objective of the present study aimed to evaluate the genetic diversity of eleven Saudi date palm cultivars using SSR markers, certain chemical compositions within these cultivars, and RPW infestation levels indices.

2. Materials and Methods

2.1. Description of the study area

Qassim province is located in the center of Saudi Arabia approximately 400 km (250 ml) northwest of the capital city, Riyadh. Qassim is considered one of the biggest agricultural areas in Saudi Arabia with about 7 million date palm trees of different cultivars. Out of those 7 million, more than 5 million date palm trees are considered fruitful trees (71.5%) (Abdel-Baky et al., 2022, 2023). Because of the diversity of palm cultivars in Qassim and the multiplicity of farms, and the fact that the farms are far away from each other, therefore, each farm is considered an independent agricultural ecosystem.

2.2. Plant materials

Mature date trees of eleven cultivars {Nabtet Ali, Shakrah, Red Sukary, Um Kobar, Saghy, Barhi, Khalas, Rashodia, Nabtet Sultan, Yellow Sokkari, and fohool (date palm tree males)}, represent the most economical value for the date cultivars in Saudi Arabia, were selected for this study. All the selected trees were almost the same age and uniform in growth. The trees were in the good physical condition and were subjected to the same horticultural management practices. Leaves samples of the selected cultivars were collected and transferred immediately to the biotechnology Lab, Plant Production, and Protection Department, College of Agric. & Vet. Med., Qassim Univ.

2.3. Monitoring program of the epidemic pest of date palm trees, R. ferrugineus

Since the monitoring procedure is a system of comprehensive periodic detection for RPW infestations and damage in each of the study areas, the direct visual examination to search for the presence of RPW symptoms was applied as the appropriate detection measurement method before the infestation of RPW escalates and spreads between areas and/or within the same date palm orchard.

Due to the seriousness and economic importance of *R. ferrugineus,* the Ministry of Environment, Water and Agriculture in Saudi Arabia has categorized RPW as a quarantine pest that affected date palm trees and restricted the transporting of offshoots and whole date palm parts between all agricultural regions within Saudi Arabia. The visual samples and the external symptoms of RPW infestation in date palm orchards were applied in this study, as well as, the aggregation pheromone traps for two successive years (2019 and 2020). Monitoring RPW presence, RPW infestations, and RPW damage were done biweekly scheduled using aggregation pheromone baited traps within 79 date palm farms (56419 trees with an average 5129 trees/ cultivar) including 11 cultivars of date palms (10 female cultivars and one male cultivar). The aggregation pheromone (Ferrolure 700 mg, ChemTica International S.A., Costa Rica) was used in the baited traps, and the pheromone was replaced monthly based on weather temperature.

2.4. Date palm trees examination

A monthly periodic visual examination was done on each date palm farm separately. A scientifically trained team performed a monthly survey at each location. Data on RPW infestation and damage were taken randomly in each location according to the status of RPW infestation and the conditions of each farm. Evidence and data were gathered with the help of teamwork from the Palm Weevil Control Center in Qassim (Abdel-Baky et al., 2022, 2023).

2.5. Determine date palm cultivars damage

The survey procedures followed by the Palm Weevil Control Center in Qassim (Ministry of Environment, Water, and Agriculture) were applied such as the visual examination methods as well as, the aggregation pheromone traps (Abdel-Baky et al., 2022, 2023). RPW adult preference was evaluated in 11 date palm cultivars under free-choice conditions in 79 date orchard farms in Qassim, Saudi Arabia. Visual examination was carried out with aid of the damage symptoms of RPW. The detailed symptoms of *R. ferrugineus* could be summarized as described by Abraham et al. (1998) as (1) the presence of a tunnel attack on the trunk and the base of the leaf petiole, (2) oozing out a thicky brown liquid from the tunnels, (3) the appearance of chewed plant tissues, with a typical fermented odor around the tunnel opening, (4) appearance of a dry branch mostly those emerging from the bases of the leaves, (5) chewing sounds by larvae, (6) trunk breaking and (7) empty pupal cocoons. From the survey results among 11 date palm cultivars from 79 date farms at Qassim, two biological criteria were applied to determine the highest and lowest infestation percentages as a natural indicator for preferring and non-preferring date palm hosts by RPW adults when choosing the best host for feeding, laying its eggs and start a new life cycle to produce a new generation. These biological parameters are a) RPW infestation and b) RPW abundance. The first one is infestation % which means the number of infested palm trees by RPW of a particular variety / divided by the total number of trees examined for the same variety. While the other criterion is abundance % which means the number of infested palm trees by RPW of a particular variety / divided by the total number of infested palm trees of all studied cultivars.

2.6. Chemical assessment (major nutritional elements analysis)

Samples of eleven date palm cultivars were picked up from different infestation levels by RPW. Chemical analysis was conducted at the Plant Production and Protection Department, College of Agriculture and Veterinary Medicine, Qassim University. The plant samples were stored at -20ºC, according to the described method by Ni et al. (2001).

2.7. Estimation of the nitrogen (N), phosphorous (P), potassium (K), and calcium (Ca) values among selected date palm cultivars

Since RPW's attraction to date palm trees depends on kairomone (kairomone is volatile emitted from the host plants that attract herbivores insects to the host plant), which may be affected by some plant nutrient elements, some of which may play as a resistance factor. Therefore, the necessary nutrients for the plant were selected, and its role in highly infected and resistant cultivars in nature was estimated. To assess N (nitrogen), P (phosphorus), K (potassium), and Ca (calcium) values in selected cultivars of the date palm leaves, samples were collected from different farms in the Qassim region. Samples were rinsed with deionized water and dried in the air for 25 min and then at 70 °C for 48 hr. All samples were sieved to <0.15 and <0.5 mm using a stainless steel mill. 0.2-gram quantities of each plant sample were digested with a mixture containing concentrated HNO_3, H_2SO_4, and H_2O_2 (Jones, 2001). In the digestion solution, calcium was measured using Atomic Absorption Spectrometry (Shimadzu 7000) while K was measured using a Flame photometer (JENWAY PFP7). Nitrogen content was measured by the Kjeldahl method (Jones, 2001), using (Buchi B-324 Kjeldahl Distillation Unit). The nitrogen in dried samples is converted to ammonium sulfate by a mixture of sulfuric and salicylic acid during digestion. Ammonia was distilled, on steam-distillation, from ammonium sulfate by concentrated sodium hydroxide and reception ammonia in boric acid. The boric acid captures the ammonia gas and forms an ammonium-borate complex. It was titrated against standard acid (Jones, 2001). Phosphorus (P) was determined colorimetrically by a Spectrophotometer (Varian Cary 50 Bio UV-Vis) (Jones, 2001). Phosphate readily reacts with ammonium molybdate in the presence of suitable reducing agents to form a blue-colored complex, the intensity of which is directly proportional to the concentration of phosphate in the solution. Finally, the intensity of the color was measured by a spectrophotometer at 650 nm.

2.8. Genetic study of date palm cultivars

2.8.1. DNA extraction from the resistant and susceptible date palm cultivars to RPW

The total genomic DNA of 11 cultivars of *P. dactylifera* was extracted using the method described by Sedra et al. (1998). The quality and quantity of the DNA were determined by using UV-Spectrophotometer at wavelengths of 260 and 280nm.

2.9. SSR (Simple Sequence Repeat) analysis

A (GA)n microsatellite-enriched library was used according to Billotte et al. (2004), and 8 nuclear simple sequence repeat (SSR) specific primer pairs developed by Akkak et al. (2009) were characterized in *Phoenix dactylifera* (Table 1). SSR analysis was run in a thermal cycler (Thermolyne Amplitron). The reaction mixture (25 µL) contained 1x PCR buffer with Mg Cl2 (50 mM K Cl, 10 mM Tris- HCl (pH 9.0), 2 mM Mg Cl2 and Triton X-100), 200 µM each of dATP, dCTP, dGTP, and dTTP, 30 ng template DNA, 25 pM of each primer and 2.5 unit of Taq polymerase. The mixtures were subjected to the following conditions: hold at 95°C for 1 min, followed by 35 cycles of 94°C for the 30s, 52°C for 60s, and 72°C for 2 min, and a final hold at 72°C for 8 min. PCR products were visualized along with a DNA marker on 2% agarose gel with 1X TAE buffer and detected by staining with the ethidium bromide.

Table 1. The sequence of SSR primers.

Primer name	Repeat motif	Primer Sequence (5'-3')	
		F: Forward	**R: Reverse**
mPdCIR010	(GA)22	F: ACCCCGGACGTGAGGTG	R: CGTCGATCTCCTCCTTTGTCTC
mPdCIR015	(GA)15	F: AGCTGGCTCCTCCCTTCTTA	R: GCTCGGTTGGACTTGTTCT
mPdCIR016	(GA)14	F: AGCGGGAAATGAAAAGGTAT	R: ATGAAAACGTGCCAAATGTC
mPdCIR025	(GA)22	F: GCACGAGAAGGCTTATAGT	R: CCCCTCATTAGGATTCTAC
mPdCIR032	(GA)19	F: CAAATCTTTGCCGTGAG	R: GGTGTGGAGTAATCATGTAGTAG
mPdCIR035	(GA)15	F: ACAAACGGCGATGGGATTAC	R: CCGCAGCTCACCTCTTCTAT
mPdCIR044	(GA)19	F: ATGCGGACTACACTATTCTAC	R: GGTGATTGACTTTCTTTGAG
mPdCIR048	(GA)32	F: CGAGACCTACCTTCAACAAA	R: CCACCAACCAAATCAAACAC
mPdCIR050	(GA)21	F: CTGCCATTTCTTCTGAC	R: CACCATGCACAAAAATG
mPdCIR057	(GA)20	F: AAGCAGCAGCCCTTCCGTAG	R: GTTCTCACTCGCCCAAAAATAC
mPdCIR063	(GA)17	F: CTTTTATGTGGTCTGAGAGA	R: TCTCTGATCTTGGGTTCTGT
mPdCIR070	(GA)17	F: CAAGACCCAAGGCTAAC	R: GGAGGTGGCTTTGTAGTAT
mPdCIR078	(GA)13	F: TGGATTTCCATTGTGAG	R: CCCGAAGAGACGCTATT
mPdCIR085	(GA)29	F: GAGAGAGGGTGGTGTTATT	R: TTCATCCAGAACCACAGTA
mPdCIR090	(GA)26	F: GCAGTCAGTCCCTCATA	R: TGCTTGTAGCCCTTCAG
mPdCIR093	(GA)16	F: CCATTTATCATTCCCTCTCTTG	R: CTTGGTAGCTGCGTTTCTTG
PDCAT1	(TC)21	F: CTGAAATCTCTGTTCAAATCCA TATTTTCTTT	R: GTTTGGATCTATTTGTGAGT
PDCAT2	TCGCTG(TC)3(TC)3T(TC) 3T (TC)3T(TC)4TTCT GTCCCG(TC) 16T(TC)	F: GGCCTTCTCTTCCCTAATGGGA	R: GTTTCTTGCCCCTGTTCTTTCCCTC
PDCAT3	(CA)8 - (GT)3(CA)4	F: CAAGGATAGGTGTGATGACCACC GCTGGAATT	R: GTTTGTCCTTTTAACTTCTT
PDCAT4	(CA)8TT(CA) 4(GA)20	F: TAACGAGTCCACACAC	R: CTGGGTAAAGCTTATAAG
PDCAT5	(AG)16	F: GGCCCGTCCTTGGATTAGAG	R: CTACGTTGTCCCGTCAATTGG
PDCAT6	(CA)14(GA)23	F: AATCAGGGAAACCACAGCCA AGCCTCAG	R: GTTTAAAGCCTTCTCAAGAT
PDCAT8	(TC)16	F: GCTTAAGTGGTTAGTTGCCAA AGTTGA	R: GTTTGGCAGAAGTATTGAAA
PDCAT10	(TC)16	F: CACTGCTCCTGTTGCCCTGT	R: TGTAGAAGGGCAGAGGACGG

2.10. Data analysis

2.10.1. Statistical analysis

RPW infestation:

The degree of RPW infestation was determined according to Facylate's equation (Facylate, 1971), in which the degree of infestation (Equation 1)

$$(\mathbf{A}) = (\mathbf{n} / \mathbf{N}) \mathbf{X} \; \mathbf{100} \quad (1)$$

Where:

A = The infestation incidence percentage.

n = Number of infested samples.

N = The total number of samples (uninfested + infested) taken on each inspection date.

The surveyed scales were classified into three categories according to their percent of infestation as follows:

- Heavy infestation when percentages are more than 50%.
- Medium infestation when percentages between 25 -50%.
- Low infestation when percentages between 1 -25%.

The statistical significance of differences between the four nutrient elements was determined using two-way ANOVA in STATISTICA for Windows (StatSoft, 2005). To measure the significant differences between four nutrition elements and cultivars to get direct comparisons among treatments' means were done by calculating the LSD (least significant difference) values. Any difference larger than the LSD was considered a significant result. As well as, the person correlation coefficient was studied to correlate the relationship between nutritional elements and cultivars.

The principal component analysis (PCA) was used to reduce the dimensionality of the dataset without losing important information. PCA-biplot extracted from PCA. Also, PCA was carried out to show multiple dimensions of the date palm cultivars in a scatter plot using the PAST software version 1.62 (Hammer et al., 2001).

2.11. Date palm tree genetic analysis

SSR data were scored for presence (1), absence (0), or a missing observation (9), and each band was regarded as a locus. Two matrices, one for each marker, were generated. The genetic similarities (GS) were calculated according to Nei and Li (1979): GS = 2Nij/(Ni+Nj), where Nij is the number of bands present in both cultivars i and j, Ni is the number of bands present in cultivar i, and Nj is the number of bands present in cultivar j. Based on the similarity matrix, a dendrogram showing the genetic relationships between cultivars was constructed using the unweighted pair group method with arithmetic average (UPGMA) through the software NTSYS-pc version 2.11 (Rohlf, 2000).

3. Results

3.1. Evaluation of date palm cultivars based on R. ferrugineus infestation percentages

The results of the survey study of the eleven date palm cultivars collected from seventy-nine farms indicate significant differences in *R. ferrugineus* infestation percentages under the free-choice trial (natural infestation) (Figure 1). The survey results showed a large discrepancy between palm cultivars in terms of the presence or absence of RPW infestation, in addition to a discrepancy between these cultivars in the degree of infestation (Figure 1). Therefore, date palm cultivars were classified into two groups based on the degree of RPW infestations.

The 1st group contains the highly susceptible cultivars to infest with RPW with high rates of infestation than other cultivars. This means that these cultivars are more favorable to RPW adults for laying eggs and the larval stages of feeding. So, these cultivars can be arranged in descending order according to the infestation degree (Abundance %) as follows: yellow Sukary, Khalas, Barhi, Rashodia, Nabtet Sultan, and fohool (date palm males).

On the contrary, the 2nd group involved only five cultivars that had the lowest RPW infestation % (resistant cultivars), three of them almost had no infestation (which means that they are not a preferred host for RPW feeding and /or egg-laying). These resistant cultivars can be arranged in ascending order (Abundance %) according to the degree of infestation to Nabtat Ali, Shakrah, Saqie, red Sukary, and um Kobar (Figures 1-2). Therefore, the following studies focus on six sensitive (susceptible) date palm cultivars and five other resistant cultivars to RPW infestation based on the criterion of infestation and abundance percentages, as indicated in the Figure 1 since the infestation % in three of the resistance cultivars was zero.

3.2. Date palm chemical assessment (major nutritional elements)

Plant minerals play an important role in the growth of plants and associated herbivore insects. Nitrogen, phosphorus, potassium, and calcium are classified as macronutrients. Data obtained in Table 2 showed variations in the four mineral values according to the date cultivars. A table showing the values of the four elements (nitrogen, phosphorous, potassium, and calcium) was estimated in 11 different date palm cultivars. Values of

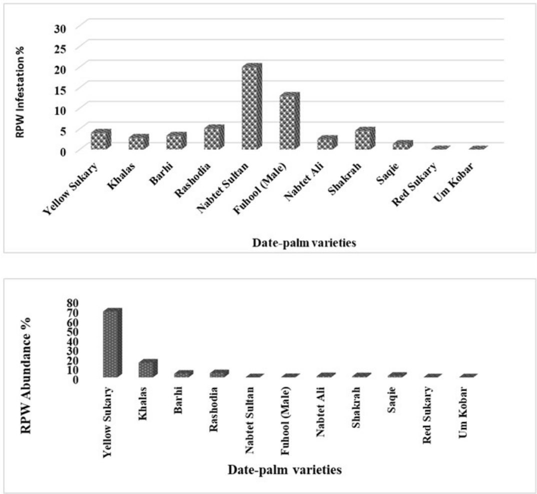

Figure 1. RPW infestation and abundance % on the susceptible and resistant date palm cultivars.

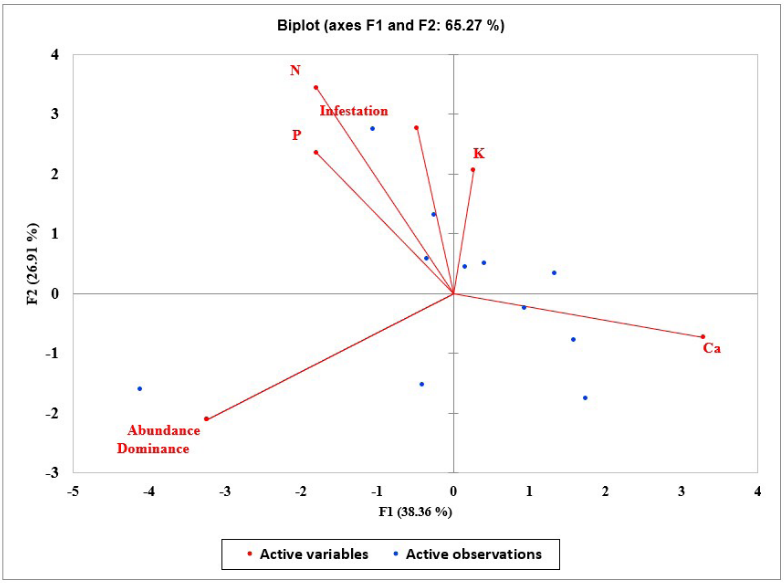

Figure 2. Biplot graphs based on the principal component (PC) analysis of parameters that measure the effect of the four nutritional minerals and RPW infestation and abundance percentages of date palm cultivars.

Table 2. Chemical analysis of four nutritional elements in 11 date palm cultivars.

Parameters / Cultivars	Amount of nutritional elements mg/Kg (% ± S.E.)			
	Nitrogen	Phosphorous	Potassium	Calcium
	N	P	K	Ca
Saqie	1.20 ± 0.0707 I*	0.07 ± 0.0005 D	0.76 ± 0.0051 I	1.36 ± 0.0051 A
Nabtet Ali	2.32 ± 0.0071 B	0.50 ± 0.0071A	0.94 ± 0.0068 G	1.20 ± 0.0103 D
Shakrah	0.90 ± 0.0051 J	0.04 ± 0.0005 E	1.04 ± 0.0071 D	1.27 ± 0.0051 C
Um Kobar	1.39 ± 0.0086 H	0.08 ± 0.0037 D	1.01 ± 0.0086 E	1.33 ± 0.0086 B
Red Sukary	1.60 ± 0.0051 G	0.07 ± 0.0051 D	1.03 ± 0.0051 D	1.12 ± 0.0051 E
Rashodia	1.72 ± 0.0071 F	0.41 ± 0.0051 B	0.90 ± 0.0068 G	0.92 ± 0.0093 G
Barhi	2.62 ± 0.0071 A	0.07 ± 0.0005 D	1.18 ± 0.0051 B	0.90 ± 0.0114 H
Khalas	1.91 ± 0.0037 D	0.36 ± 0.0071 C	0.90 ± 0.0051 G	0.90 ± 0.0068 H
Nabtet Sultan	0.77 ± 0.0071 K	0.07 ± 0.0005 D	0.80 ± 0.0071 H	0.84 ± 0.0051 I
Fohool (Male)	2.12 ± 0.0051 C	0.05 ± 0.0005 E	1.13 ± 0.0045 C	0.97 ± 0.0051 F
Yellow Sukary	1.82 ± 0.0068 E	0.05 ± 0.0007 E	1.28 ± 0.0071 A	0.67 ± 0.0071 J
Average	1.67 ± 0.075	0.16 ± 0.022	1.00 ± 0.021	1.04 ± 0.029
L.S.D. at 0.05 level	0.063**	0.011**	0.017**	0.021**
C.V.%	2.96	5.5	1.33	1.61

C.V. = coefficient of variation. *Means for each upper case letter followed by the same letter in the same coulum are not significantly different at P < 0.05. ** Highly significant at $P \leq 0.01$.

nitrogen ranged from 0.77± 0.0071 mg/Kg in the Nabtet Sultan to 2.62 ± 0.0071 in the Barhi. While phosphorous values ranged from 0.04 ± 0.0005 mg/Kg in the Shakrah to 0.50 ± 0.0071 in the Nabtet Ali. Potassium recorded the lowest value (0.76 ± 0.0051 mg/Kg) with the Saqie and the highest one (1.28 ± 0.0071 mg/Kg) with the Yellow Sukary. On the other hand, values of calcium varied also based on the cultivar, which listed the lowest value (0.84 and 0.67 mg/Kg) with Nabtet Sultan and Yellow Sukary, respectively, and the highest value (1.36 ± 0.0051 mg/Kg) with the Saqie (Table 2).

3.3. The relationship between the four nutritional minerals and RPW infestation

The correlation coefficient was used to assess the relationship between the four important minerals and the degree of infestation by RPW (Table 3). The Simple linear correlation uses to measure the degree to which two variables vary together or measure the intensity of the association between two variables. This correlation was tested to see if the r value was significantly different from zero, which means there is an association between the two evaluated variables.

RPW infestation and abundance % had a positive correlation relationship with both nitrogen and phosphorus with r values listed as 0.041 and 0.123 for abundance and 0.228 and 0.542 for infestation, respectively. Meanwhile, r values had a negative slight relationship with potassium with records of -0.158 and -0.041 for abundance and infestation, respectively. In contrast, the value of r was negatively high with abundance and calcium content (-0.585) (Table 3). Regarding the resistant cultivars, r values were positive but lower with nitrogen and phosphorous and had the highest positive values with potassium and calcium.

3.4. Principle Component Analysis (PCA) of date palm cultivars

The principal component analysis (PCA) revealed that the four nutritional minerals and RPW infestation and abundance percentages were highly affected by the cultivars of date palm (Figure 2). The first two components of the PCA accounted for 65.27% of the total variance, whereas PC1 accounted for 38.36% and PC2 accounted for 26.91%. It was noted that calcium content and abundance percentage were negatively correlated (Figure 2). Interestingly, the PCA biplot revealed that (the major contributors of resistant characteristics) calcium content and RPW abundance percentage were strongly associated with resistance date palm cultivars of cluster A. The dendrogram was confirmed by PCA. Date palm cultivars in the PCA scatter plot (Figure 3), indicated by the ellipses numbered A, B, C, D, and E, seemed to form a very close grouping in the dendrogram (Figure 3). Interestingly, the highest sensitive (susceptible cultivar) to RPW (Yellow Sokary) was in separate group E. Moreover, the results indicated that the resistance cultivars of date palms (Nabtat Ali, Shakrah, red Sukary, Saqie, and um Kobar) were clustered in group A (Figure 3).

3.5. Genetic polymorphism in date palm genotypes using SSR markers

24 SSR primers pairs of Billotte et al. (2004) and Akkak et al. (2009) were tested for their ability to generate

Table 3. General correlation matrix (Pearson (n-1) between the four nutritional minerals and RPW infestation and abundance percentages.

Variables	Abundance	Infestation	Dominance	N	P	K	Ca
Abundance	**1**	-0.107	**1.000**	0.041	0.123	-0.158	**-0.585**
Infestation	-0.107	**1**	-0.107	0.228	0.542	-0.041	-0.030
Dominance	**1.000**	-0.107	**1**	0.041	0.123	-0.158	**-0.586**
N	0.041	0.228	0.041	**1**	0.415	0.558	**-0.625**
P	0.123	0.542	0.123	0.415	**1**	-0.287	-0.352
K	-0.158	-0.041	-0.158	0.558	-0.287	**1**	-0.030
Ca	**-0.585**	-0.030	**-0.586**	**-0.625**	-0.352	-0.030	**1**

Values in bold are different from 0 with a significance level of alpha=0.05.

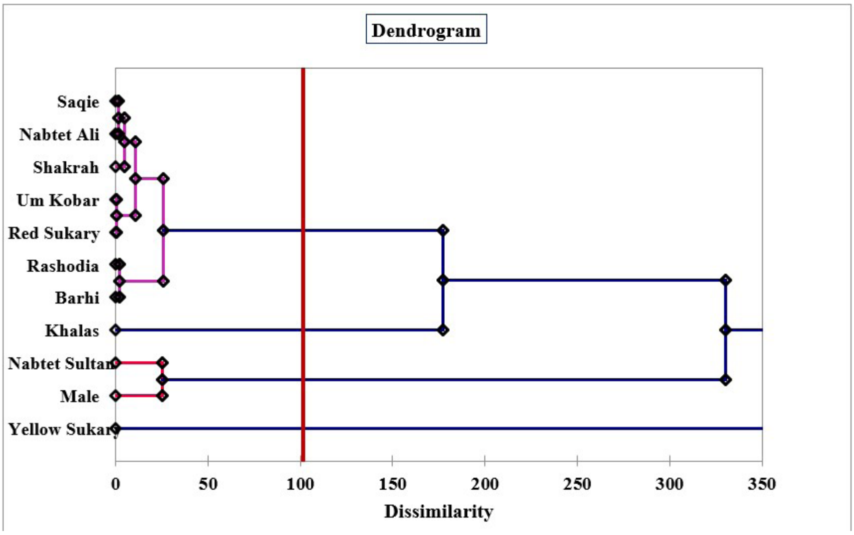

Figure 3. UPGMA dendrogram for 11 date palm cultivars. Group A indicates 5 resistant cultivars, while groups B, C, D, and E indicate to six susceptible cultivars.

expected SSR banding patterns in Saudi date palms at the DNA level (Table 1). A total of 14 primers showed polymorphic bands among 11 cultivars (10 female and one male) successfully (Table 4). Twenty-four bands have been generated and showed polymorphism among the date palm cultivars. Each primer gave 1 to 4 bands. The results indicated that double base primer (GA) 19 (mPdCIR032), and the SSR primers PDCAT4 and PDCAT6 gave 3 polymorphic bands with 100% polymorphism. While the SSR primer PDCAT3 gave two polymorphic bands among date palm cultivars (Figure 4).

The genetic distances between date palm cultivars were studied by the mentioned 14 primers (Table 4). The genetic cluster tree, using the UPGMA program, divided the genotypes into 2 main groups with similarity coefficients between 0.56 and 0.91 (Figure 5). The 1st main group included the genotypes; Nabtet Ali, Red Sukary, Um Kobar, and Shakrah at similarity coefficients between 0.56. Interestingly, this group was the most resistant palm cultivars to RPW. The 2nd main group included the genotypes; Saqie, Barhi, Khalas, Rashodia, Nabtet Sultan, and Yellow Sokary at similarity coefficients between 0.69 and 0.86. The second group was divided into two subgroups. The first sub-group included the cultivar Yellow Sokary at similarity coefficients of 0.75 and 0,91. In addition, this cultivar was the highest sensitive (susceptible cultivar) to RPW. The second sub-group included the genotypes; Saqie, Barhi, Khalas, Rashodia, and Nabtet Sultan. Also, these cultivars were sensitive (susceptible cultivars) to RPW.

4. Discussion

In Qassim Saudi Arabia, *Rhynchophorus ferrugineus* infestation % varied from one year to another which was relatively higher in 2016 (2.24%) and 2017 (3.19%), then gradually decreased to reach its lowest in 2020 (0.73%) and varied among the eight study locations, reaching

PDCAT3

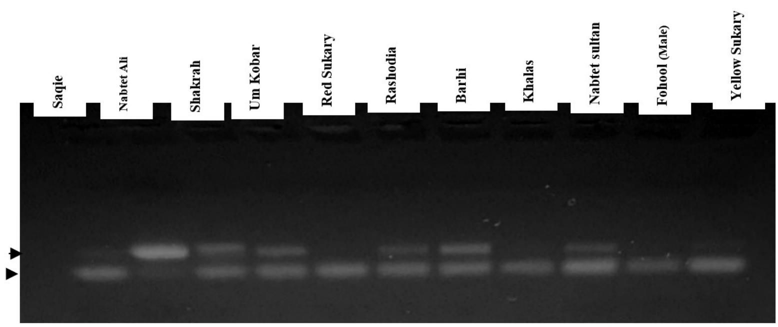

Figure 4. Agarose gels showing restriction patterns of PCR-amplified SSR primer (PDCAT3). Arrows indicate the polymorphic bands between date palm cultivars.

Table 4. SSR (microsatellites) markers used for polymerase chain reaction (PCR) screening of date palm genomic DNA.

NO	Primer	Repeat Motifs/ folds	Total	Polymorphic	Monomorphic	% Polymorphic
1	mPdCIR010	GA)22	**2**	**1**	**1**	**50**
2	mPdCIR016	(GA)14	**2**	**1**	**1**	**50**
3	mPdCIR025	(GA)22	**1**	**1**	**0**	**100**
4	mPdCIR032	(GA)19	**3**	**3**	**0**	**100**
5	mPdCIR035	(GA)15	**2**	**2**	**0**	**100**
6	mPdCIR085	(GA)29	**2**	**2**	**0**	**100**
7	mPdCIR090	(GA)26	**2**	**1**	**1**	**50**
8	mPdCIR093	(GA)16	**3**	**1**	**2**	**33**
9	PDCAT2	TCGCTG(TC)3(TC)3T(TC) 3T (TC)3T(TC)4TTCT GTCCCG(TC) 16T(TC)	**1**	**0**	**1**	**0**
10	PDCAT3	(CA)8 - (GT)3(CA)4	**2**	**2**	**0**	**100**
11	PDCAT4	(CA)8TT(CA) 4(GA)20	**3**	**3**	**0**	**100**
12	PDCAT5	(AG)16	**2**	**1**	**1**	**50**
13	PDCAT6	(CA)14(GA)23	**3**	**3**	**0**	**100**
14	**PDCAT8**	**(TC)16**	**4**	**3**	**1**	**75**

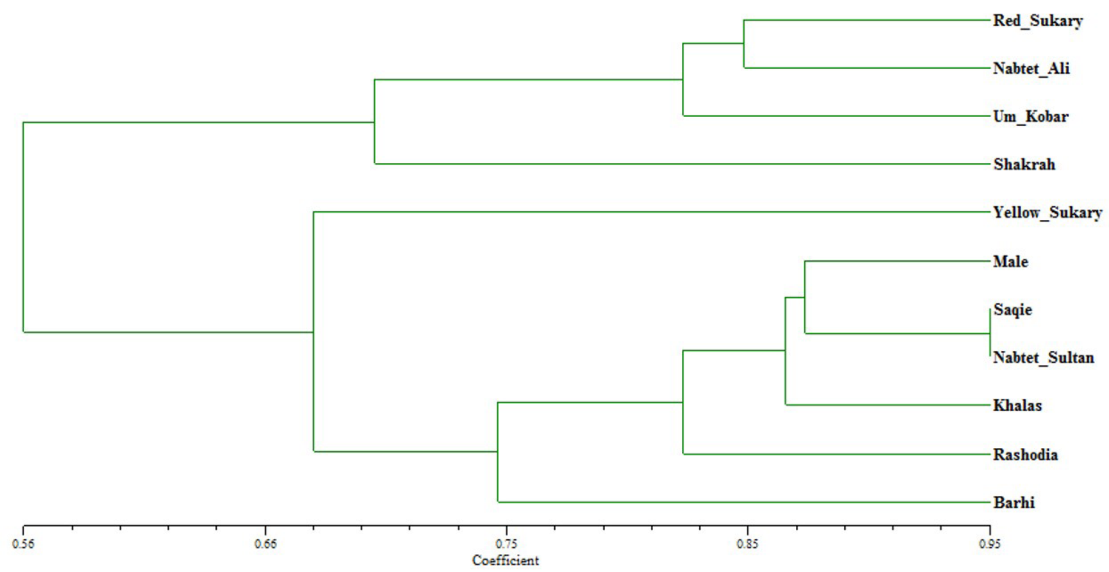

Figure 5. Dendrogram constructed from similarity coefficients and the clustering of date palm cultivars using SSR markers.

the highest percentage in location G (SA7) with a general average of 4.31%, maybe due to the management protocol applied by the Ministry of Environment, Water, and Agriculture (Abdel-Baky et al., 2022, 2023). Meanwhile, the effectiveness of the control methods increased sharply concerning the treated palms percentage rising from 52.141% in 2015 to 90.0% in 2020 with a general average of 72.73% (Abdel-Baky et al., 202, 2023).

IPM has several definitions that can be generally classified into two basic approaches, the 1st one focuses on the judicious use of chemical pesticides informed by the pest monitoring system and determined the economic decision levels, and the 2nd one is considered the more comprehensive form which emphasizes on agroecosystem component before intervention with reactive pest control options (Bajwa and Kogan, 2002; Zalucki et al., 2009). On the other hand, IPM involves the use of adaptations in the plant hosts (morphologically, chemically, and/or genetically) to avoid or tolerate insect pests which are called plant resistance (PR) (War et al., 2012; Anderson et al., 2019). In this study, the results revealed that the resistant cultivars can be arranged in ascending order (Abundance %) according to the degree of infestation to Nabtat Ali, Shakrah, Saqie, red Sukary, and um Kobar. Host plant resistance (HPR) can be benefits from the fact that all plant hosts (either wild or/and cultivated) have several adaptive characteristics which can protect themselves from herbivores' harmful insects such as the red palm weevil, *R. ferrugineus*. Plant resistance (means) is a group of plants that have natural defense mechanisms such as being unpalatable to eat, or drawback insect growth through scarcity of the necessary nutrients, or a plant becomes immune (Bajwa and Kogan, 2002; Zalucki et al., 2009; War et al., 2012; Anderson et al., 2019). These adaptive characteristics can be used to protect our strategic crops from harmful insect damage in agricultural systems. Therefore, the majority of cultivated date palm varieties are genetically homozygous which may be a practice to reduce genetic diversity among species and increases the likelihood of infestation by economically important insect pests such as *R. ferrugineus* (Elshibli and Korpelainen, 2011; Brown and Caligari, 2011; Birch et al., 2012). Thus, IPM practices helped to re-establish eco-friendly defense mechanisms against pests in plants, like plant resistance mechanisms (Anderson et al., 2019). Plant-resistant defense mechanisms involve escape in space and time, including incompatible biological associations between the plant and an insect pest, or creating physicochemical barriers and accommodation by replacement or repair of damaged plant parts in an ecologically compatible manner (Eyles et al., 2010). So, plant resistance has a vital role in trying to enhance environmental stability in economic crops. Plant hosts varied in the quality of their nutritional content to harmful insects, maybe due to a primary metabolite level, or the quantity and nature of the secondary metabolite (Soufbaf et al., 2010, 2012). The differences among the cultivars of host quality include differences in morphological features, nutritional contents, and secondary metabolites' concentrations (Soufbaf et al., 2012). Consequently, host plant quality can affect several biological characteristics of a harmful insect by impairing growth, lowering pest resistance, and reducing fecundity (Price et al., 1980).

Concerning the chemical composition of nutrient mineral elements and their effects on date palm cultivars and associated insects, nitrogen is considered a very important required mineral for the growth of insects (Rostami et al., 2012; Bala et al., 2018). Clancy (1995) hypothesized that plant nitrogen may be responsible to determines the amount of food ingested, which affects the amounts of other nutrients consumed. So, a proper balance of many nutrients appears to be the most important factor in the nutritional ecology of insect herbivores. Kolsi-Benzina and Zougari (2008) found that in the cultivar Deglet Nour the highest N, P, K, and Ca leaflets contents were 1.6, 1.26, 0.27, and 0.41, respectively. Also, El-Shurafa (1984) determined N, P, K, and Ca % in parts of the palm and found the highest and lowest value of N was 1.16 and 0.185 in rachis and pinnae, respectively. Concerning P, the highest and lowest value of P was 0.125 and 0.024 in seed and rachis, respectively. Regarding K, the highest and lowest value of K was 1.10 and 0.07 in stalk and rachis, respectively. Also, the highest and lowest value of Ca was 0.533 and 0.125 in pinnae and seed, respectively.

Phosphorus and potassium levels are related to aphid infestation that may cause resistance or susceptibility to plants. Phosphorus contributes to decreasing the host's suitability to insect pests by changing secondary metabolites such as phenolics and terpenes. Increasing phenolics (tannin, lignin) acts as antifeedant or toxic substances that affect negatively insect presence (Facknath and Lalljee, 2005). Increasing the phosphorus treatment reduced the population of aphids. The population decreased significantly with an increase in the rate of application while increasing the phosphorous level to increase the response of other insect pests such as *Empoasca* sp. and *Frankliniella occidentalis* (Bala et al., 2018). According to Marschner (2012), calcium is one of the necessary elements for plants, which is considered an essential element in the composition of the middle plate where calcium is responsible for holding together the cell walls of plants. Strengthening the cell wall, which helps to stabilize the plant and prevents many pests from feeding and entering the cell. The lack of calcium weakens the plant, especially the cell walls and their cohesion, which exposes plants to infection with pests and diseases. In addition, the deficiency of Ca leads to the leakage of metabolic products that stimulate pathogen infections easily (Spann and Schumann, 2009). The calcium concentration in the plant samples ranged between 0.67 and 1.4%. They demonstrated also high levels of potassium and calcium help in plant resistance which has a negative sign on the building up of aphid populations. High levels of potassium help in plant resistance enhance secondary compound metabolism and reduce the accumulation of carbohydrates (Kapoor et al., 2020) that protect the plant from pest attacks. Deficiency symptoms of potassium such as thin cell walls, weakened stalks and stems, smaller and shorter roots, accumulation of sugar in the leaves, and accumulation of unused N encourage disease infection (Graham, 1983). Each of these factors minimizes the ability of the plant to resist entry of infection by fungus, bacteria, and viruses. K fertilization is widely reported to affect insect infestation and disease incidence in many host plants (Marschner, 1995). Potassium provides high resistance against insect pests, while phosphorus decreases the plant's suitability to various insect pests and calcium elements also reduce the pest populations (Khattab, 2007; Bala et al., 2018). All four fertilization elements had indirect effects by changing the nutrient components of the date palm, which had an effect on the state of the date palm's resistance to RPW or not. Therefore, the recommended use of more healthful date palm fertilizing elements to be stimulating the development of techniques to increase the resistance against RPW, as well as the other date palm insects, will be recommended. consequently, our study suggests that the deleterious and beneficial effects of high nutrient concentrations on red palm weevil should receive further attention and that the interactive, indirect effects of nutrient additions on RPW performance should be studied.

The variation in the responses of palm cultivars to palm weevil infestation (including sensitive and resistant ones) may be attributed to many factors, such as the chemical composition of nutrient mineral elements in the soil or the genetic diversity among the cultivated varieties. The differences in nutritional elements components between date palm varieties may be due to several factors, such as characteristics variations of the soils because the samples were taken from different cultivation areas representing the Qassim region. The other factor may have resulted in the genetic difference between date palm varieties under the study.

Regarding the genetic diversity among the cultivated varieties, there is an appreciated resemblance between our results as compared with previous studies. Faqir et al. mentioned that only two microsatellite markers mpdCIRO25 and mpdCIRO85 produced polymorphic bands within the expected range (Faqir et al., 2016). In this study,14 primers showed polymorphic bands (24 bands) among 11 date palm cultivars (10 female and one male) successfully. Therefore, 24 polymorphic markers were used for the assessment of genetic relationships in date palm cultivars. The genetic diversity among Saudi date palm cultivars, using the UPGMA program, divided the genotypes into 2 main groups with similarity coefficients between 0.56 and 0.95. The study of Khierallah et al. (2011) concluded that the genetic distance among Iraqi date palm cultivars varied from 0.171 to 0.938 indicating diverse relationships.

The dendrogram based on combined nutritional minerals and RPW infestation and abundance percentages data (Figure 3) clustered the resistance cultivars of date palm (Nabtat Ali, Shakrah, red Sukary, and um Kobar) in one cluster, these cultivars were grouped in one cluster from the rest of the cultivars in the dendrogram based on molecular data. The highest sensitivity (susceptible cultivar) to RPW (Yellow Sokary) was clustered in separate clusters based on nutritional minerals and RPW infestation and abundance percentages data, and SSR markers. Al-Ayedh (2008) showed that the cultivar 'Yellow Sukkary' proved to be the best overall host for rearing red date palm weevil among the cultivars tested. Therefore, the dendrogram formed by SSR markers showed a similar relationship among date palm cultivars as shown in the score plot of the principal component analysis (Faqir et al., 2016).

A great amount of information is generated about red palm weevil biology that was accumulated over time; however, this data has not been analyzed in conjunction with the areas of knowledge mentioned above (date palm genetic diversity and role of nutritional elements and their role in hampering RPW development and damage). The analysis of such data considering the holistic view of bioecology and nutrition (nutritional ecology) certainly will generate questions about developing an IPM strategy for RPW, whose answers are currently undetermined so far.

Our results generated many questions that should be taken into consideration in the future in the field of red palm weevil control, as follows: What are the effects of intra- and interbreeding among date palm cultivars on RPW biology and the crop? How are the RPW feeding behavior and physiology affected by a change in the host quality (nutritional and genetic) over time? How do date palm fertilizers regimes effects RPW feeding, reproduction, and dispersion? Which factors make changes in RPW status over time and that will help the emergence of new haplotypes and species of RPW to become more dangerous than now? These and many other questions that are generated from this study and others previously carried out research should be analyzed and answered considering RPW paradigm bio-ecology and date palm nutrition and its genetic variation status. This information will not only help to understand the response of *Rhynchophorus* species against the variety of control methods but will also yield data to generate holistic IPM programs.

Therefore, the integration between genetic diversity, chemical structures, and RPW infestations rates promoted the understanding of the interplay between the diversity of RPW management (short-time scale), and the resistance genes, plant nutrition, and dynamics of the diversity of RPW through domestication and diversification (long-timescale). However, most of the commercial date palm cultivars have lost some defensive traits against pests through selective breeding for yield during domestication. Further, a scheme that outlines strategies and approaches for prebreeding and their introgression into elite cultivars for developing RPW-resistant date palms must be proposed as a possible way forward in the future. Thus, there is a strong need for the development of cost-effective and biologically based integrated pest management (IPM) practices including host-plant resistance (HPR).

5. Conclusions

The prospect of using resistant markers from date palm varieties against palm weevils (*Rhynchophorus* species) could add another way to value, screen, and characterize date palm varieties for conservation and use. Because of their broad distribution of date palm varieties and wild relatives are good materials for applying useful genetic variants associated with controlling harmful insects and diseases. Our results proved that genetic diversity in date palm cultivars could enhance the capacity for detecting patterns of geographic structure in Saudi Arabia. Another important finding was detecting the chemical composition of nutrient mineral elements associated with specific RPW infestation. Calcium content was negatively correlated with RPW abundance. Ultimately, understanding the mechanisms that the genetic variation using SSR markers and calcium content of date palm cultivars could help to predict responses of RPW populations to future new IPM era on insect control.

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