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Effect of glutathione (GSH) on Date palm (*Phoenix dactylifera* L.) micropropagation

Ahmed Madi Waheed Al-Mayahi*, Osama Nazim Jafar, Khaun Ali Mohsen

Date Palm Research Centre, University of Basrah, Basrah, Iraq

Abstract

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The investigation was carried out to evaluate the influence of glutathione (GSH: levels 0, 0.1, 0.5 1.0, and 2.0 mM) on the callus growth, shoot multiplication and phytochemicals of *in vitro* shoots of the Date palm cv. Barhee. The optimum concentration of GSH was 1.0 mM. Such concentration improved the callus growth and increased its weight to 312.0 mg, the addition of this substance of the same concentration level showed the highest response rate and the number of shoots per jar (73.34 ± 2.69 and 8.83 ± 0.80 shoots/jar, respectively). Also, the 1.0 GSH application resulted in reducing the percentage of browning to 0.0 compared to the other concentrations. The results of the biochemical analysis revealed that treatments GSH of 0.1, 0.5 and 1.0 mM reduced the total soluble phenols compound (TSPC). These treatments were also more effectively reducing peroxidase (POD) and phenylalanine ammonia-lyase (PAL) activity than the concentrations of 2 mM or 0 (control treatment).

Keywords

antioxidant substances, browning, phenols compound, phenylalanine ammonia-lyase (PAL), shoot regeneration

Introduction

Date palm (*Phoenix dactylifera* L.) of the family Arecaceae is one of the most critical economic fruit crops. The origin of the Date palm is unknown exactly, but it is believed that it originated in southern Iraq at least 6,000 years ago (Al-Yahyai and Manickavasagan, 2012). Date palm is propagated through the use of seeds or offshoots, but both methods suffer from some inadequacies and restrictions (Al-Khateeb, 2006). Therefore, it has become necessary to propagate the Date palm using alternative biotechnology methods. Indeed, Date palm micropropagation enables rapid and large-scale multiplication of uniform plants that are pathogen-free and without pests during plant material exchange, and true-to-type (Al-Mayahi, 2015). The culture medium is a vital ingredient of the plantlets' growth response that provides most of the requirements for growth and development (Jasim et al., 2009; Ibrahim et al., 2013; Al-Mayahi, 2019a). Optimization of the composition of the growth media for micropropagation is fundamental due to the nutrition requirements of the plants. The γ-glutamylcysteinyl glycine it's called glutathione and too GSH-reduced ($C_{10}H_{17}N_3O_6S$) is a water-soluble tripeptide that possesses active thiol compound that contributes to cell division and organ differentiation in most plant species (Vieira et al., 2012; Fraga et al., 2016). Usually supplied culture media with the GSH is used to enhancing cell regeneration and the development of immature somatic embryos (Stasolla et al., 2004). The different roles and functions of antioxidants, including glutathione, open vast possibilities to use them to promote tissue culture and plant regeneration. As well as the decisive role of GSH in regulating organogenesis, regeneration, and differentiation of cultures propagated *in vitro* (Belmonte

*Corresponding author:
e-mail: hng_1988@yahoo.com

et al., 2006; Tyburski and Tretyn, 2010) plays a role. Therefore, in this investigation, we concentrate on the attempt to evaluate the effects of glutathione, as one of the most effective antioxidants, which is supplemented in the MS culture media, and its role in developing a possible improved protocol for propagation of Date palm cv. Barhee *in vitro.*

Materials and methods

Plant and culture establishment

Callus tissues induced from the apical buds of the Date palm cv. Barhee were separated and cultured on a medium composed of (MS) (Murashige and Skoog, 1962) (4.4 g ml^{-1}), with additional 10 mg l^{-1} Naphthalene Acetic Acid (NAA), 2 mg l^{-1} 2iP and 3 g l^{-1}, 100 mg l^{-1} glutamine, 5 mg l^{-1} thiamine HCl, 1 mg l^{-1} biotin, 30 g l^{-1} sucrose, and solidified with agar at 7.0 g l^{-1} and 1.5 g l^{-1} activated charcoal. After getting the sufficient amount of callus, we transferred it to MS medium with the same composition mentioned previously, excluding growth regulators combination (0.5 mg l^{-1} NAA, 0.5 mg l^{-1} BA, 0.5 mg l^{-1} kinetin (K)), and 0.5 g l^{-1} activated charcoal (Al-Mayahi, 2016), suitable for shoot regeneration.

To study the effects of glutathione on the multiplication of buds and changes in phytochemicals, MS culture media were equipped with different concentrations of GSH (0, 0.1, 0.5, 1.0, and 2.0) mM l^{-1}. The pH of the medium was adjusted between 5.7–5.8 with 0.1 N NaOH or HCl before the addition of agar. Sterilization of the culture's jars with media was performed by autoclaving at 121 °C and pressure of 1.04 kg cm^{-2} for 20 min. The cultures were incubated under room temperature 25 ± 2 °C, with a 16 h photoperiod provided by white fluorescent lamps. The results of the experiments evaluated after 12 weeks of culture callus on the media were: percentage of shoot regeneration and shoot numbers.

Browning percentage (%)

The browning percentages were scored according to the following: - No shoot browning, + Moderate shoot-browning, +++ Severe shoot-browning.

Total soluble phenols compound (TSPC)

TSPCs were estimated with the Folin-Ciocalteu reagent according to the method described by Sadasivam and Manickam (1996). The absorbance temperature (650 nm) was measured by using a spectrophotometer; results were expressed as (mg GAE/g).

POD and PAL assay

Peroxidase (POD) was measured as described by Diaz et al. (2001). Phenylalanine ammonia-lyase (PAL) activity was assayed following the method of (Syklowska-Baranek et al., 2012).

Statistical analysis of data

Data from each experiment were subjected to One-way Analysis of Variance (ANOVA). A completely random CRD was used. The GenStat software was applied. All significant means were separated using the least significant difference ($P < 0.05$).

Results

Effect of various concentrations of glutathione on some growth criteria

Callus growth

According to the results obtained in Fig. 1, glutathione at (0.1, 0.5, and 1.0) mM was found suitable for producing callus. However, the weight of callus was decreased with an increase in the concentration of glutathione to 2 mM in the culture media (Fig. 2e). The weight of callus was increased in the media containing glutathione at 1.0 Mm, which was 312.0 mg. This value was higher than that in the media supplemented with the other of glutathione concentrations. The statistical analysis shows significant differences between 2.0 Mm of GSH and control (no additives) treatment on callus weight at the 0.05 level (Fig.1 and Fig. 2a, e).

The percentage of indirect multiplication and shoots production

The cultured buds showed significant variations in their response percentage, as well as the formation of shoots, under the influence of different concentrations of GSH (Fig. 3). Tissues cultured in medium containing GSH at 1.0 mm showed a better response rate (73.34 ± 2.69%) of buds producing shoots and average shoots number (8.83 ± 0.80 shoots/jar) compared to tissues cultured in the medium containing 2 mM GSH which recorded the lowest response rate with the lowest number of shoots which were 33.34% and 3.97 ± 0.81 shoots, respectively (Table 1, Fig. 3e).

Browning percentage

The results in Fig. 2e showed that the treatment of GSH at 2.0 mM resulted in severe browning in most cultures +++. Control treatment (0.0 GSH) showed a moderate browning rate (+) (Fig. 2a), while the browning response was not recorded in the GSH treatments at 0.1, 0.5 and 1.0 Mm (Fig. 2b, c, and d).

Impact of glutathione on some biochemical traits

Total soluble phenols compound (TSPC)

The data in Fig. 4 show that the optimal treatment was observed in the medium containing 0.1, 0.5, and 1.0 mM of glutathione, which showed significant superiority in reducing the content of the cultures of total soluble phenols compound compared with other treatments. It also showed the results of biochemical analyses that phenolic compounds were increased with increasing levels of glutathione to 2 mM, where it reached 1.53 mg

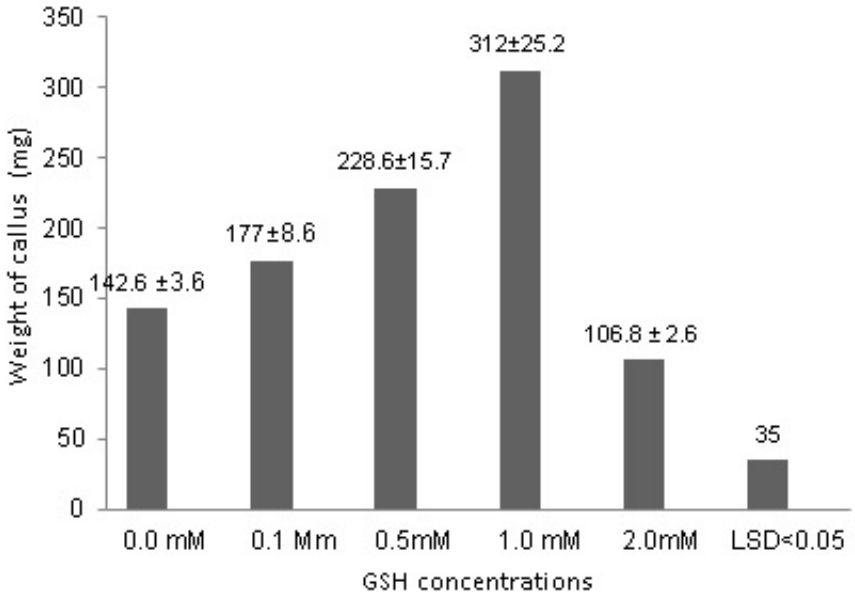

Fig. 1. Effect of different glutathione (GSH) concentrations on the weight of callus (mg) in the Date palm cv. Barhee in vitro (± Standard error).

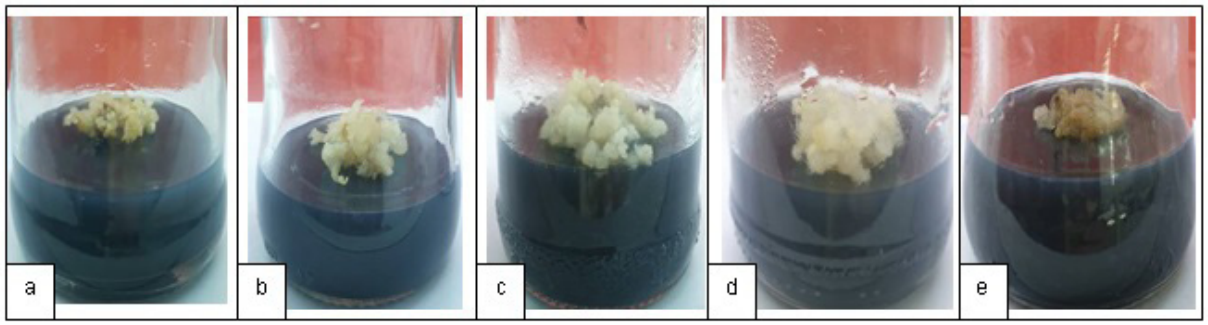

Fig. 2. Callus proliferation on MS medium with (a) control (0.0 mM GSH), (b) 0.1 mM GSH, (c) 0.5 mM GSH, (d) 1.0 mM GSH, (e) 2.0 mM GSH treatments.

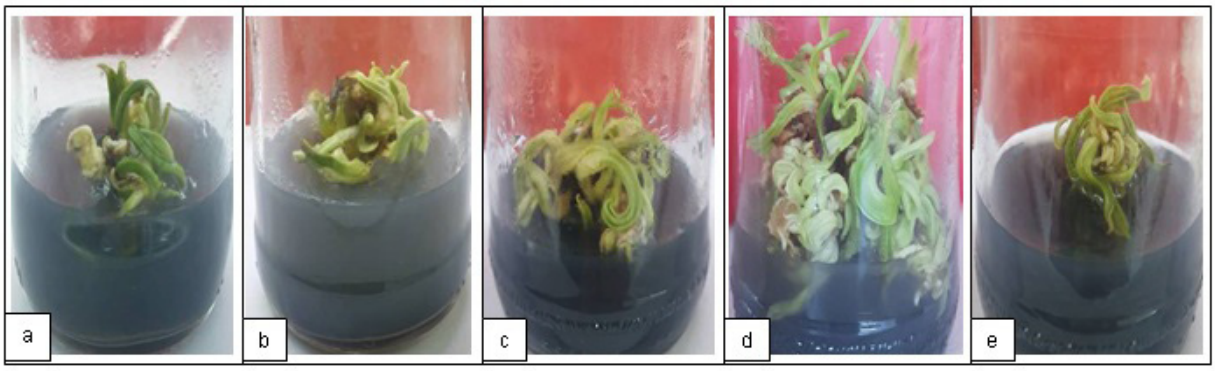

Fig. 3. Effect of different concentrations of glutathione (GSH) on the bud induction in the Date palm callus cv. Barhee:(a) control (0.0 mM GSH), (b) 0.1 mM GSH, (c) 0.5mM GSH, (d) 1.0mM GSH, (e) 2.0 mM GSH.

GAE/g. Results also showed the accumulation of phenolic compounds reached 1.35 mg GAE/g at the control treatment.

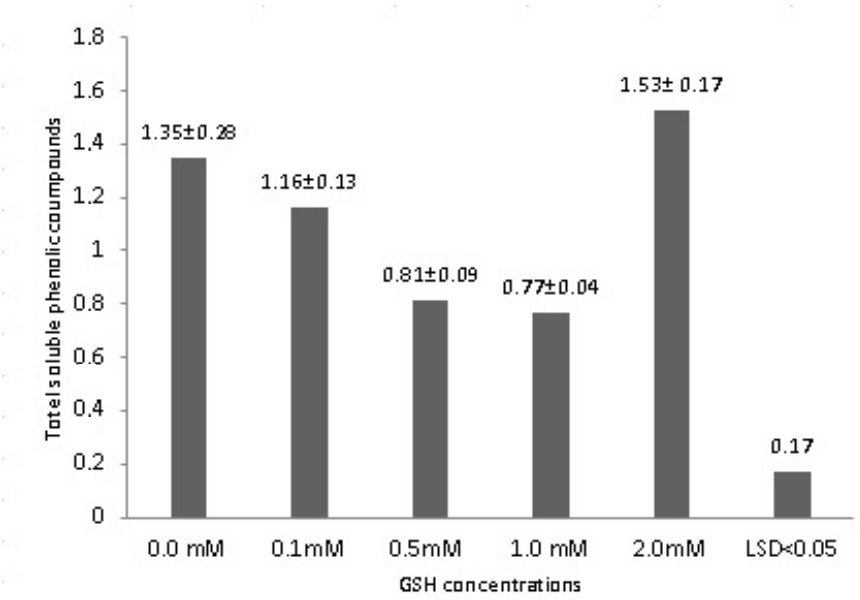

Fig. 4. Effect of GSH concentration on total soluble phenols compounds of Date palm tissues cv. Barhee (± Standard error).

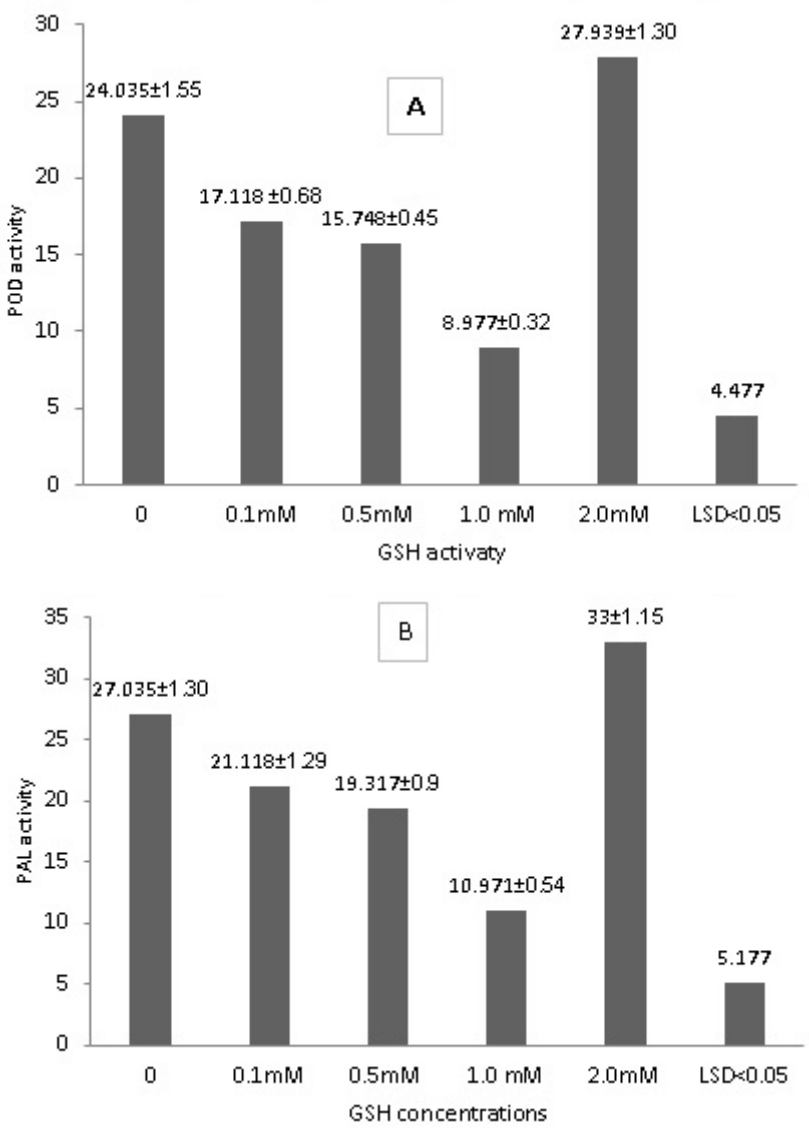

Fig. 5. Effect of glutathione (GSH) concentrations on activity of (a) peroxidase (POD), (b) phenylalanine ammonia-lyase (PAL) in in vitro cultured Date palm cv. Barhee (± Standard error).

Table 1. Effect of glutathione (GSH) on the response percentage of callus for shoot regeneration and number of shoots (± Standard error)

Treatments (mM)	Response percentage (%)	No. of shoots / jar
0.0 control	40.00 ± 1.53 *c	4.53 ± 0.84c
0.1	53.34 ± 1.81 b	6.50 ± 0.47 b
0.5	60.00 ± 1.85 b	6.93 ± 0.58 b
1.0	73.34 ± 2.69 a	8.83 ± 0.80 a
2.0	33.34 ± 1.20 c	3.97 ± 0.81 c
LSD P < 0.05	13.33	1.07

*Different letters indicate statistically significant differences between means.

Peroxidase (POD) and phenylalanine ammonia-lyase (PAL) activity

The statistical analysis showed that the treatment of GSH at 2.0 mM GSH recorded the highest activity of both POD and PAL (27.94 and 33.0%) respectively, which was significantly different compared to the activity of the above-mentioned antioxidant enzymes at other treatments ($p < 0.05$) (Fig. 5a, and b). While the lowest activity of antioxidant enzymes, POD and PAL were achieved at treatment 1.0 mM GSH, which were 8.977, and 10.71 respectively.

Discussion

The results of this study showed that the gradual increase in the concentration of GSH to 1.0 mM in the culture medium enhanced good response, but at higher levels (1.0 mM) significantly depressed the shoot proliferation. Cultures on growth medium containing 1.0 mM GSH stimulated callus proliferation, which reflected positively on the increase its weight. The different biochemical properties of GSH enable the potential to participate in plant growth and development where the addition of glutathione (GSH) to the growth media improved shoot organogenesis. These data suggest that the process of shoots regeneration is dependent on optimal GSH levels in the culture media and that the mechanism of inhibition of shoots growth by GSH may occur as a result of the accumulation of supraoptimal GSH levels in the cultured tissues. Tissue analysis demonstrated that the presence of GHS is necessary for the persistence of cell divisions (Vernoux et al., 2000). GSH was found in cells that are characterized by their active division. At the same time, it was not present in cells that are characterized by their slow division, which indicates that developing tissues require glutathione (Tyburski and Tretyn, 2010). Providing the media with GSH contributed to stimulating growth when applied with levels that suit the physiological levels. Our results are in harmony with those obtained by Belmonte et al. (2006), Tyburski and Tretyn (2010), who reported the critical role of GSH in organogenesis and differentiation *in vitro*. One of the most significant constraints facing the *in vitro* tissue of the Date palm is the browning that causes tissue damage a few days after cultivation (Al-Mayahi et al., 2010; Al-Mayahi, 2014). Browning is a significant obstacle to the successful exploitation of these vital trees (Al-Mayahi, 2018 and 2019b). Browning of tissues occurs as a result of increased production of TSPCs and their consequent oxidation by the activity of polyphenol oxidase (PPO). Guan et al. (2019) mentioned that PAL is the main enzyme that participates in the first step for the biosynthesis of phenylpropanoid. Through these results, a positive relationship can be observed in the activity of PAL and TSPCs, indicating an increase in the content of TSPCs due to increased PAL activity. As Andersone and Ievinsh (2002) reported that the POD and PAL oxidizing enzymes are the stimulants of biosynthesis of polyphenols, as well as they contribute to browning due to wounds, these enzymes are an indicator of oxidative metabolism. In this study, we observed a decrease in PAL and POD activity in GSH-treated cultures accompanied by reduced TSPCs. Many previous studies indicated that GSH has the potential for practical use to control TSPCs (Tabiyeh et al., 2006). Glutathione plays major regulatory roles in the process of the cell cycle progress within the meristems, as it is participating in the redox-dependent determination of regeneration and quiescence patterns (Tyburski and Tretyn, 2010). Tabiyeh et al. (2006) reported that the treatment with GHS led to decrease phenylalanine ammonia-lyase PAL activity, which may be caused by a reduction in secondary metabolism production and the browning of tissues in favour of primary metabolism processes supporting the increase of growth. Browning tissue discoloration was associated with oxidative stress which was also supported by a higher content of TSPCs (Misra et al., 2010). Therefore, GSH can be usefully used to decrease the browning of tissue. The low activity of antioxidant enzymes resulting from treatment with antioxidants confirms the conclusions of Tabiyeh et al. (2006), who concluded that GSH reduced the enzymes' antioxidant activity.

Conclusions

From the results of this study we concluded that treatment with different concentrations of glutathione (0.1, 0,5 and 1.0) mM had a significant effect on most of the studied traits, the results showed that inhibit peroxidase (POD) and phenylalanine ammonia-lase activity (PAL), it was associated with inhibiting the production of TSPCs, it also coincided with the promotion of callus growth and buds development and reduced the browning of tissues cultured as compared to the concentration of 2.0 GSH and 0.0 (control treatment). 1.0 mM GSH showed its superiority in all studied traits.

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