ABSTRACT

Pollination-induced senescence in *Dendrobium* Pompadour flowers was investigated. Pollinated flowers were held in distilled water and in treatment solutions containing either sucrose, glucose, aminooxyacetic acid (AOA) or silver thiosulphate (STS) while unpollinated flowers were held in distilled water. Morphological changes were observed visually while physiological parameters investigated included ethylene production, colour change, petal thickness, fresh weight, dry weight and water uptake. Biochemical changes determined were starch, total sugar and reducing sugar, cell wall hydrolases, total protein, soluble and insoluble protein. Furthermore, 1D SDS PAGE was also carried out to profile the protein changes in all the flowers. Finally five genes; 1-aminocyclopropane-1-carboxylate oxidase (*ACCO*), 1-aminocyclopropane-1-carboxylate synthase (*ACCS*), ethylene receptor 1 (*ETR1*), ethylene response sensor 1 (*ERS1*) and ethylene response sensor 2 (*ERS2*) were isolated and amplified via RT-PCR. Characterization of the isolated genes was carried out using a number of bioinformatic tools.

Pollinated flowers held in distilled water demonstrated accelerated senescence which was categorized into five different stages from the point where the flower was fresh and open to reaching full closure (termination of vase life) and finally showing the first sign of necrosis (advanced stage of senescence). The morphological changes corresponded to a climacteric pattern of ethylene production with an ethylene peak detected on the day. In contrast ethylene was not detected in unpollinated flowers. Vase life of pollinated flowers held in distilled water was terminated on the 2nd day and reached advanced stage of senescence on the 7th day. Unpollinated flowers stayed fresh throughout the experiment as well as pollinated flowers held in 0.05 mM AOA and 0.6 mM STS. Vase life of flowers held in sugars was terminated on the 4th day. Accelerated physiological

changes were also observed in pollinated flowers whereas treated flowers demonstrated a delay in the changes. Flowers held in 0.05 mM AOA and 0.6 mM STS showed similar trends of physiological changed with that of unpollinated flowers.

Carbohydrate changes in pollinated flowers held in distilled water included a decline in starch and non-reducing sugars and an increase in total and reducing sugars. In unpollinated flowers and pollinated flowers held in 0.05 mM AOA and 0.6 mM STS, starch was retained while little change was observed in the status of sugars. Similar trends were observed in pollinated flowers held in 2% sucrose and 4% glucose at the initial days of observation but subsequently emulated the trend displayed by pollinated flowers held in distilled water. Measurement of cell wall hydrolases on PG, PL, PME and cellulose showed a more pronounced role for PG, PL and cellulose during pollination-induced senescence. The rate of activity for these hydrolases increased upon pollination whereas PME activity was rather low. Membrane deterioration determined by measuring electrolyte leakage was also accelerated by pollination and was found to be delayed in pollinated flowers held in treatment solutions.

Total protein content in pollinated, unpollinated and treated pollinated *D.* Pompadour flowers exhibited a general trend of decline towards the end of the experiment. However, pollinated flowers held in distilled water showed a more rapid decline and recorded the lowest amount of total protein at the end of the experiment. Profile analysis using 1D SDS PAGE showed that three polypeptides were regulated throughout the process, which was identified as PR-like protein, SAM synthase and seed storage protein. Amongst the three proteins, the most abundant protein expressed was the PR-like protein followed by SAM synthase and the seed storage protein. The PR-like protein and SAM synthase significantly increased following pollination.

All physiological parameters investigated indicated that pollination induced a series of deteriorative processes that ultimately results in the death of the perianth. The effectiveness of ethylene inhibitors in circumventing the pollination effects established the major role of ethylene in regulating the post-pollination symptoms. The inability of sugars to overcome the pollination affects as effective as ethylene inhibitors suggests that sugars may play a role secondary to that of ethylene.

Finally, the *ACCO* and *ACCS* genes isolated from pollinated flowers were comparable to the genes deposited in the GenBank in terms of size and contains the signature domains that are pivotal for the genes to function. Partial sequences were obtained for *ETR1*, *ERS1* and *ERS2*. Nevertheless, they contain the signature domain of ethylene receptors. All genes isolated showed strong homology to the corresponding gene family in the database. Three dimensional (3D) structure of the genes was also predicted and phylogenetic trees were constructed to show the relationship of the genes with other orchids or flowers. These are the first genes to be isolated in *D.* Pompadour and will be deposited into the GenBank.

ABSTRAK

Kelayuan kelopak bunga yang disebabkan oleh pendebungaan telah dikaji. Bunga yang tidak didebungakan diletakkan di dalam air suling manakala yang telah didebungakan diletakkan di dalam air suling dan juga larutan rawatan yang mengandungi samada glukosa, sukrosa, *aminoxyacetic acid* (AOA) atau *silver thiosulphate* (STS). Perubahan morfologi telah direkod secara visual manakala perubahan fisiologi yang dikaji termasuk perubahan warna bunga, ketebalan kelopak, berat segar dan kering serta penyerapan air. Perubahan biokimia yang dikaji adalah kandungan kanji, jumlah gula, gula penurun dan bukan penurun, enzim dinding sel, jumlah protein, protein larut dan tak larut. Analisa *1D SDS PAGE* turut dijalankan untuk mengenal pasti profil perubahan protein dalam kesemua bunga yang dikaji. Lima gen; *ACCO, ACCS, ETR1, ERS1 and ERS2* telah diasingkan dan ciri-ciri gen tersebut telah dikaji menggunakan program bioinformatik.

Bunga yang didebungakan dan diletakkan dalam air suling menunjukkan kelayuan yang lebih awal dan boleh dikategorikan kepada lima peringkat, bermula dari bunga yang kembang sepenuhnya dan segar kepada bunga yang layu dan nekrotik. Perubahan-perubahan morfologi adalah selari dengan penghasilan gas etilina yang tertinggi pada hari pertama. Sebaliknya, gas etilina tidak dikesan dalam bunga yang tidak didebungakan. Jangka hayat bunga yang didebungakan berakhir pada hari kedua dan menunjukkan tahap kelayuan *termaju* pada hari ketujuh. Bunga yang tidak didebungakan serta dirawat dengan larutan 0.05 mM AOA dan 0.6 mM STS kekal segar sepanjang eksperimen. Jangka hayat bunga yang diletakkan dalam larutan 2% sukrosa dan 4% glukosa pula berakhir pada hari keempat. Perubahan fisiologi yang dipercepatkan turut berlaku dalam bunga yang didebungakan manakala bunga yang dirawat menunjukkan perubahan yang lebih perlahan. Bunga yang diletakkan dalam

larutan 0.05 mM AOA dan 0.6 mM STS menunjukkan simptom yang sama dengan bunga yang tidak didebungakan.

Dari segi perubahan karbohidrat, bunga yang didebungakan dan diletakkan dalam air suling menunjukkan penurunan dalam jumlah kanji dan gula penurun manakala jumlah gula dan gula penurun meningkat. Bunga yang tidak didebungakan dan bunga yang didebungakan serta dirawat dengan 0.05 mM AOA dan 0.6 mM STS mengandungi kanji yang lebih tinggi selain perubahan yang kecil dalam status gula. Bagi bunga yang dirawat dengan 2% sukrosa dan 4% glukosa~~,~~ pada akhir pemerhatian perubahan yang berlaku menyerupai bunga yang didebungakan dan disimpan dalam air suling. Kajian ke atas enzim *PG, PL, PME* dan *cellulase* menunjukkan peranan yang lebih besar dimainkan oleh *PG, PL* dan *cellulase* sewaktu kelayuan disebabkan pendebungaan. Kadar aktiviti bagi ketiga-tiga enzim tersebut meningkat selepas pendebungaan sementara aktiviti *PME* adalah rendah sepanjang pemerhatian. Kemerosotan membran yang ditentukan dengan mengukur kebocoran elektolit turut dipercepatkan oleh pendebungaan manakala larutan rawatan melambatkan proses kemerosotan membran.

Jumlah protein dalam bunga yang didebungakan, tidak didebungakan dan dirawat secara amnya menunjukkan penurunan. Walaubagaimanapun, bunga yang didebungakan dan diletakkan di dalam air suling menunjukkan penurunan yang paling besar dan jumlah protein yang terendah. Analysis profil protein menggunakan kaedah *1D SDS PAGE* menunjukkan tiga polipeptida yang dikawalatur sepanjang proses tersebut, dan dikenalpasti sebagai *PR-like protein*, *SAM synthase* dan *seed storage protein*. Di antara protein-protein tersebut, protein yang mempunyai ekspresi yang tertinggi adalah *PR-like protein* diikuti oleh SAM *synthase* dan *seed storage protein.*

PR-like protein dan *SAM synthase* menunjukkan peningkatan yang signifikan selepas pendebungaan.

Kesemua parameter fisiologi yang dikaji menunjukkan bahawa pendebungaan menyebabkan berlakunya proses-proses kemerosotan yang berakhir dengan kematian bunga. Keupayaan antagonis etilina dalam menghalang kesan-kesan pendebungaan membuktikan kepentingan etilina dalam mengawalaturkan simptom-simptom lepas pendebungaan. Kesan gula yang kurang efektif dalam mengatasi kesan pendebungaan menunjukkan bahawa gula berkemungkinan memainkan peranan yang tidak sepenting etilina dalam mengawalatur pendebungaan.

Akhir sekali, gen *ACCO* dan *ACCS* yang diasingkan daripada bunga yang telah didebungakan mempunyai persamaan dengan gen-gen yang terdapat dalam *Genbank* dari segi saiz dan turut mempunyai domain *signature* yang penting untuk fungsi gen-gen tersebut. Jujukan separa juga didapati untuk *ETR1,ERS1*dan *ERS2*. Jujukan-jujukan tersebut turut mengandungi domain *signature.* Kesemua gen menunjukkan homologi yang tinggi dengan gen-gen dalam pangkalan data. Struktur 3D dan analisa filogenetik juga telah dibina untuk menunjukkan perhubungan gen-gen tersebut dengan orkid dan bunga yang lain. Gen-gen ini adalah yang pertama kali diasingkan daripada *D.* Pompadour dan akan dimasukkan dalam *GenBank.*

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ACCO	1-aminocyclopropane-1-carboxylate (ACC) oxidase
ACCS	1-aminocyclopropane-1-carboxylate (ACC) synthase
AOA	aminooxyacetic acid
AVG	aminoethoxyvinylglycine
BLAST	basic local alignment search tool
bp	base pairs
CAPS	3-[cyclohexylamino]-1-propanesulfonic acid
CDNA	complementary deoxyribonucleic acid
dH_2O	distilled water
DNA	deoxyribonucleic acid
DW	dry weight
EDTA	ethylenediaminetetraacetic acid
EIN	ethylene insensitive
ETR	ethylene receptor
ERS	ethylene response sensor
FW	fresh weight
KAc	potassium acetate
kDa	kilo Dalton
LiCl	lithium chloride
1-MCP	1-Methylcyclopropane
NCBI	National Centre for Biotechnology Information
PAGE	polyacrylamide gel electrophoresis
PCD	programmed cell death
PCR	polymerase chain reaction

PG	polygalacturonase
PIPS	pollination induced petal senescence
PL	pectate lyase
PME	pectin methyl esterase
PVP	polyvinylpyrrolidone
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
SDW	sterile distilled water
STS	silver thiosulphate
TEMED	tetramethylethylenediamine
Tris-HCl	tris hydrochloride

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