The Effects of Gibberellic Acid on Sex Expression and Secondary Sexual Characteristics in Papaya

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Abstract. The vegetative forms of male (XY), female (XX), and hermaphrodite (XY^h) papaya (*Carica papaya* L.) plants are phenotypically identical. However, the flower and inflorescence morphology of each sex type is unique. Gynodioecious varieties SunUp, SunUp Diminutive mutant, and dioecious AU9 were used to test the response of papaya to gibberellic acid (GA₃). Exogenous applications of GA₃ on female and hermaphrodite flowers of papaya did not yield any sex reversal phenotype but caused a significant increase in peduncle elongation and inflorescence branch number in all treated plants. An increase in flower number was seen in females but not hermaphrodites or males. There was an increase in plant height for all treated plants except SunUp Diminutive mutant, suggesting that the mechanism causing the dwarf phenotype is independent of gibberellins. Gibberellin metabolism genes were identified in the papaya genome, none of which mapped to the sex-determining region of either the male- or hermaphrodite-specific region of papaya Y or Y^h chromosome. We hypothesize that a transacting regulatory element that enhances gibberellin biosynthesis plays a role in the extreme length of the male papaya peduncle.

Papaya (*Carica papaya* L.) is a major fruit crop in tropical and subtropical regions. In a comparison against 34 commonly consumed fruits, papaya ranks number one in many categories including vitamin A and C, potassium, folate, niacin, thiamine, riboflavin, iron, and calcium (Liebman, 1992). Papaya is a trioecious species with a pair of nascent sex chromosomes controlling sex type; females are XX, males XY, and hermaphrodites XY^h (Ming et al., 2001, 2007). The sex-determining region of the sex chromosomes comprises ≈8.1 Mb and 3.5 Mb of the Y- and X-specific region of the Y/Y^h and X chromosome, respectively (Liu et al., 2004; Wang et al., 2012). Although sex is determined by the sex chromosomes, environmental factors

such as temperature and nutrient availability can alter sex expression in papaya (Ghosh and Sen, 1975; Lange, 1961). The sex-determining region contains not only the genes controlling sex type (male and female sterile genes), but also genes controlling sex-linked traits such as peduncle length and inflorescence branch number.

The vegetative forms of the three sex types of papaya are phenotypically homomorphic but the reproductively mature trees are morphologically distinct. Male trees develop long, pendulous inflorescences (60 to 90 cm) bearing numerous flowers that originate at the leaf axis. The individual male flowers are slender and tubular in shape with five stamens surrounding a small vestigial ovary. The extreme length of the primary peduncle is a principal attribute of male papaya plants. Female and hermaphrodite flowers also develop from the leaf axis but, unlike male flowers, they are borne singly or in small cymes of up to three flowers on short peduncles (0 to 4 cm). Pistillate flowers have an enlarged base, taper toward the tip, are much larger than staminate or hermaphroditic flowers, and lack any trace of stamens. Hermaphrodite flowers are less bulbous than female flowers but thicker than

male flowers. They are intermediate in size, have functional carpels and stamens, and are able to self-pollinate. Hermaphrodite plants are often preferred for commercial production because they do not require males for pollination and produce pyramidal-shaped fruits, which are good for packaging compared with the spherical fruit produced by females (Higgins and Holt, 1914).

Plant hormones play diverse roles in growth and development with most having pleiotropic effects. Gibberellin is a major phytohormone that functions to not only promote normal growth and development, but it is also able to affect sex expression in many plant species (reviewed by Khryanin, 2002). Although more than 100 GAs have been identified in plants, only a few are known to function as biologically active hormones, including GA₁, GA₃, GA₄, and GA₇. Most GAs exist in plants as either precursors to bioactive GAs or as deactivated GAs. Bioactive GA concentration can vary between tissue types and is determined by the rate of synthesis vs. the rate of deactivation.

GAs were first recognized as a result of their effects on stem elongation. Exogenous applications of GA₃ reversed the mutant dwarf phenotype of peas (Brian and Hemming, 1955) and maize (Phinney, 1956), allowing them to reach mature heights similar to normal plants. GAs are now known to be essential in many diverse stages of plant growth and development, including germination, leaf expansion, flower induction and development, and fruit and seed growth (Davies, 1995). GAs have also been shown to promote the formation of males flowers in many dioecious species, including *Spinacia oleracea* (Chailakhyan and Khryanin, 1978a), *Cannabis sativa* (Chailakhyan and Khryanin, 1978b; Mohan Ram and Jaiswal, 1972), *Rumex acetosella* L. (Bavrina et al., 1991), and the gynodioecious line of *Cucumis sativus* (Mitchell and Wittwer, 1962; Peterson and Anghder, 1960; Pike and Peterson, 1969). Although GA usually promotes maleness in flowers, this is not true for all species. Exogenous applications of GA₃ in maize results in the feminization of tassels at the apical inflorescence (Hansen et al., 1976).

The present study was designed to identify GA metabolism genes in papaya and to examine the effects of GA₃ on sex expression and the development of female and hermaphrodite plants. We also examined the effect of exogenous GA₃ applications to determine if the dwarf phenotype of SunUp Diminutive mutant plants was the result of a mutation in GA metabolism, GA perception, or an unrelated pathway.

Materials and Methods

Field treatment

Expt. 1. Seeds of three papaya cultivars—AU9, SunUp, and SunUp Diminutive—were sown in the experimental field station at Kunia, Oahu, HI. The plants were clearly marked before treatment began to observe any changes in sex types or development.

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Treatment began 16 weeks after germination and continued twice a week for 2.5 weeks for a total of five treatments. Exogenous application of 3.5 mL of 0.1 mM GA₃ (Sigma Aldrich, St. Louis, MO) was applied with a spray bottle to newly emerged leaf tissue [AU9 (n = 18), SunUp (n = 16), SunUp Diminutive (n = 11)]. Four days after the last treatment, the following data were recorded: plant height, peduncle length, inflorescence branch number, flower number, and sex type. Plant height was taken from the ground to the top of the newly emerged leaf. Peduncle length was measured from the trunk of the plant to the beginning of the first flower. The total number of inflorescence nodes and branches was also recorded. Sex type was established by examining flower structure and determining the presence/absence of carpals and stamens. The control papayas were left to grow under natural conditions in the field (no application of GA₃). Experimental groups were compared using the Student's *t* test. Values were considered significant if $\alpha \leq 0.05$.

Expt. 2. Seeds of AU9 were sown in the experimental field station at Kunia, Oahu, HI. Male and female AU9 papaya plants were treated with GA₃ after 8 weeks of growth. One of three different concentrations of the phytohormone was applied—1× (125 mg·L⁻¹), 2× (250 mg·L⁻¹), or 4× (500 mg·L⁻¹) [GA₃ treatment: male (n = 17), female (n = 14)]. The solutions were applied in the same manner as described in Expt. 1 biweekly for 6 weeks. The control papayas were left to grow under natural conditions in the field (no application of GA₃). Experimental groups were compared using analysis of variance. Values were considered significant if $P \leq 0.05$.

Identification and mapping of GA metabolism genes

The *Carica papaya* SunUp genome, papaya expressed sequence tag, and papaya gene model database was screened using TBLASTN+2.2.28 against known GA metabolism genes in *Arabidopsis thaliana*, *Oryza sativa*, and *Populus trichocarpa* for transcript unit identification using default parameters. Gene structure was manually verified for exon and intron placements. Each predicted transcript was translated into all six reading frames to distinguish protein-coding genes from pseudogenes. Putative functions of protein-coding transcripts were predicted using conserved domains and homologous gene functions. Putative transcripts with truncated or missing conserved domains were not considered functional GA metabolism genes. The predicted genes were mapped to linkage groups on the simple sequence repeat genetic map (Chen et al., 2007) using blastn.

Results

Influence of GA₃ treatment on sex expression. Individual papaya trees were clearly marked at the beginning of the treatments. Flowering had already started in most of the plants and the sex was determined before the first treatment. There was no change in sex

expression in any of the GA₃-treated and untreated papaya cultivars (data not shown).

Influence of GA₃ treatment on secondary sexual characteristics. Exogenous applications of GA₃ increased peduncle length in female, hermaphrodite, and male plants in all varieties tested (Fig. 1). Female AU9 peduncles increased from 1.9 cm to 25.2 cm with GA₃ application and male AU9 peduncles increased from 11.7 cm to 31.3 cm ($P < 0.0001$). GA₃ treatment to female and hermaphrodite SunUp plants increased peduncle length from 1.2 cm to 20.0 cm and 1.1 cm to 18.8 cm, respectively ($P < 0.0001$). Female

and hermaphrodite SunUp Diminutive mutant peduncles increased from 1.5 cm to 12.2 cm and 2.1 cm to 5.9 cm, respectively ($P < 0.0003$) (Fig. 1A). The extended peduncle phenotype seen in GA₃-treated female and hermaphrodite plants is similar to untreated males (Fig. 2).

There was a marked increase in the number of inflorescence branches in all GA₃-treated plants except for the SunUp Diminutive mutant. Average AU9 inflorescence branch number increased from three to 20.2 and one to 4.3 in females and males, respectively, with the application of GA₃ ($P < 0.01$) and SunUp inflorescence branch number increased from

Fig. 1. Comparison of sex-related phenotypic traits in response to gibberellic acid (GA₃) applications. All measurements shown are means $\pm$ SE. Treatment and control plants were compared using a Student's *t* test. Values were considered significant if $\alpha \leq 0.05$. (A) Effect of GA₃ treatment on increased peduncle length, (B) branch number, (C) flower number, and (D) plant height.

Fig. 2. Morphological effects of gibberellic acid (GA_3) treatment on peduncle length and inflorescence branch number. (A) Female untreated plant, (B) female GA_3 -treated plant, (C) hermaphrodite untreated plant, (D) hermaphrodite GA_3 -treated plant, (E) male untreated plant.

Fig. 3. Effect of increasing gibberellic acid (GA_3) concentration on plant height and node number. Control plants received no exogenous applications. The treated plants were sprayed with the following concentrations of GA_3 : 1x (125 mg·L⁻¹), 2x (250 mg·L⁻¹), 4x (500 mg·L⁻¹). Values shown are means $\pm$ SE.

two to 20.25 and 3.6 to 23.6 in females and hermaphrodites, respectively ($P < 0.0001$) (Fig. 1B). Although there was a significant increase in the number of inflorescence branching in the treated plants, the number of flowers per plant remained relatively stable. Treated female AU9 and SunUp plants showed a slight increase in average flower number ($P < 0.03$), whereas male AU9 and hermaphrodite SunUp and SunUp Diminutive plants displayed no significant change in flower number between treated and control plants (Fig. 1C).

Influence of GA_3 treatment on plant height. The height of the plants rose dramatically with the treatment of GA_3 for all plants except SunUp Diminutive mutant (Fig. 1D). The treated AU9 female plants had an average height increase of 18% and AU9 male plants increased 19% compared with SunUp female and hermaphrodites, which displayed an average increase of 41% and 26%,

respectively. Increasing the concentration of GA_3 exaggerated the effect it had on the height of both female and male AU9 plants ($P < 0.0001$) but did not change the number of branching nodes per plant (Fig. 3), indicating that the increased height is the result of internode elongation rather than an increase in total number of branches and nodes.

Identification and mapping of GA metabolism genes. The major GA metabolism genes were identified in papaya using homology and mapped to supercontigs and, where possible, individual chromosomes. None of the genes mapped to the sex-determining region of the Y or Y^h chromosome (Table 1). The total number of genes involved in GA metabolism is similar between papaya and *Arabidopsis*, but if comparing the number of genes within each enzyme family, there is a considerable difference (Fig. 4). There are single copies of *ent-copalyl diphosphate synthase* (CPS) and *ent-kaurene synthase* (KS) in both *Arabidopsis*

and papaya. Papaya has appreciably more copies of *ent-kaurene oxidase* (KO) and *ent-kaurenoic acid oxidase* (KAO), three and five respectively, compared with *Arabidopsis*, which has one and two copies (Fig. 4). The opposite trend is observed in the family of soluble 2-oxoglutarate-dependent dioxygenases (2ODD)—*GA20-oxidase* (GA20ox), *GA 3-oxidase* (GA3ox), and *GA 2-oxidase* (GA2ox). *Arabidopsis* has almost twice the number of enzymes for each family compared with papaya. GA20ox and GA3ox are involved in forming bioactive GAs, whereas GA2ox functions to deactivate bioactive GAs.

Discussion

Papaya genetics, sex determination, and sex-linked characteristics associated with the three sex types have been extensively studied (Ghosh and Sen, 1975; Hofmeyr, 1938; Lange, 1961; Story, 1953; reviewed by Ming et al., 2007). Story originally proposed in 1953 that the male peduncle length is a pleiotropic effect of unit factor alleles and lies within the sex-determining region on the papaya sex chromosome. A long peduncle confers a reproductive advantage to males by increasing the number of flowers and the amount of pollen produced per tree compared with hermaphrodites. This is of particular importance in the ecology of papaya and the evolution and establishment of dioecy and sex chromosomes. Selective pressure favors a higher output of pollen for males to remain competitive against hermaphrodites (Charlesworth and Charlesworth, 1978).

Exogenous applications of GA_3 had a positive effect on peduncle length and inflorescence branch number in female and hermaphrodite papaya suggesting an increase in bioactive GAs plays a role in the extreme length and branch number seen in male papaya plants. If the short peduncles and few branch number found in females and hermaphrodites were the result of an ability to perceive GAs, exogenous GA_3 treatment would have had no effect in peduncle length and branching. GA_3 treatment on male plants exaggerated the long peduncle phenotype seen in untreated plants. The gene responsible for the peduncle length and branch number should be found only on the Y chromosome and not the Y^h or X. Mapping of GA metabolism genes yielded no mapping to the sex-specific region of the X or Y/Y^h chromosome (Wang et al., 2012). The annotated sex-specific region of the papaya sex chromosomes does not contain any known genes related to GA metabolism, perception, or signal transduction (Wang et al., 2012). This suggests that the gene responsible for the male secondary sexual characteristics is not a GA metabolism gene, but rather a transacting regulatory element.

The male-specific region of the Y and Y^h chromosome is ≈ 8.1 Mb (Wang et al., 2012) and are 98.8% similar with most of the differences resulting from repetitive element accumulation (Yu et al., 2008). Comparative genomic analysis between the sex-determining

Table 1. Gibberellin metabolism genes in *Carica papaya* and *Arabidopsis thaliana*.

Enzyme	Coding gene	Chromosome	Cp code	Coding gene	Chromosome	AGI code
CPS	<i>CpCPS</i>	3	evm.TU.supercontig_52.39	<i>AtCPS</i>	4	At4g02780
KS	<i>CpKS</i>	N/A	evm.TU.supercontig_596.3	<i>AtKS</i>	1	At1g79460
KO	<i>CpKO1</i>	N/A	evm.TU.supercontig_357.4	<i>AtKO</i>	5	At5g25900
	<i>CpKO2</i>	N/A	evm.TU.supercontig_357.5			
	<i>CpKO3</i>	N/A	evm.TU.supercontig_357.6			
KAO	<i>CpKAO1</i>	3	evm.TU.supercontig_133.12	<i>AtKAO1</i>	1	At1g05160
	<i>CpKAO2</i>	3	evm.TU.supercontig_133.24	<i>AtKAO2</i>	2	At2g32440
	<i>CpKAO3</i>	8	evm.TU.supercontig_189.35			
	<i>CpKAO4</i>	8	evm.TU.supercontig_189.36			
	<i>CpKAO5</i>	8	evm.TU.supercontig_189.37			
GA 20-oxidase	<i>CpGA20ox1</i>	9	evm.TU.supercontig_4.180	<i>AtGA20ox1</i>	4	At4g25420
	<i>CpGA20ox2</i>	5	evm.TU.supercontig_18.153	<i>AtGA20ox2</i>	5	At5g51810
	<i>CpGA20ox3</i>	N/A	evm.TU.supercontig_823.1	<i>AtGA20ox3</i>	5	At5g07200
				<i>AtGA20ox4</i>	1	At1g60980
				<i>AtGA20ox5</i>	1	At1g44090
GA 3-oxidase	<i>CpGA3ox1</i>	7	evm.TU.supercontig_23.150	<i>AtGA3ox1</i>	1	At1g15550
	<i>CpGA3ox2</i>	6	evm.TU.supercontig_91.24	<i>AtGA3ox2</i>	1	At1g80340
				<i>AtGA3ox3</i>	4	At4g21690
				<i>AtGA3ox4</i>	1	At1g80330
GA 2-oxidase	<i>CpGA2ox1</i>	5	evm.TU.supercontig_9.2	<i>AtGA2ox1</i>	1	At1g78440
	<i>CpGA2ox2</i>	5	evm.TU.supercontig_18.97	<i>AtGA2ox2</i>	1	At1g30040
	<i>CpGA2ox3</i>	4	evm.TU.supercontig_77.98	<i>AtGA2ox3</i>	2	At2g34555
	<i>CpGA2ox4</i>	9	evm.TU.supercontig_166.2	<i>AtGA2ox4</i>	1	At1g47990
				<i>AtGA2ox6</i>	1	At1g02400
				<i>AtGA2ox7</i>	1	At1g50960
				<i>AtGA2ox8</i>	4	At4g21200

Cp = *Carica papaya*; AGI = Arabidopsis Genome Initiative; CPS = *ent*-copalyl diphosphate synthase; KS = *ent*-kaurene synthase; KO = *ent*-kaurene oxidase; KAO = *ent*-kaurenoic acid oxidase; N/A = not applicable.

region in the Y and Y^h should reveal a small number of candidate genes responsible for the peduncle length and inflorescence branch number. Ethyl methanesulfonate (EMS) mutant studies have already been done in papaya with many male plants displaying shortened peduncles (Ming, unpublished data). Interestingly, mutants with shortened peduncles do not show a decrease in branching number. This is most likely the result of separate genes controlling peduncle length and branch number.

Exogenous applications of GA₃ causes rapid, intensive growth in dwarf pea plants and maize, allowing them to reach the height of normal tall-growing varieties (Brian and Hemming, 1955; Chailakhyan, 1979; Phinney, 1956). This phenomenon is not true for all mutant dwarf phenotypes (Mori et al., 2002; Tanabe et al., 2005). GA₃ treatments had a positive effect on papaya plant growth in all varieties studied except for the SunUp Diminutive mutant. The dwarf mutant did respond to GA₃ treatments in terms of peduncle length and inflorescence branch number, suggesting the short stature is likely not the result of insufficient GA biosynthesis or an inability to perceive GAs, but rather the result of a mutation independent of GA metabolism or perception.

Three distinct classes of enzymes mediate the GA metabolism pathway. The terpene cyclase class is composed of two members, CPS and KS (Aach et al., 1997; Helliwell et al., 2001; Sun and Kamiya, 1994, 1997). CPS represents the first committed step in GA biosynthesis and the enzyme is highly conserved across species. The second class of enzymes includes the cytochrome P450 mono-oxygenases, KO and KAO. GA20ox, GA3ox, and GA2ox are soluble 2-oxoglutarate-dependent dioxygenases (2ODD) and comprise the final class of enzymes. Unlike the terpene

cyclases and P450s, the 2ODD are encoded by multigene families. Inactivation of bioactive GAs is mediated by several methods, but the best studied reaction is mediated by GA2ox and involves 2 β -hydroxylation. Deactivation of bioactive GAs has recently been shown to also occur through methylation of bioactive GAs (Varbanova et al., 2007), 16 α , 17-epoxidation (Zhu et al., 2006), and conjugation (Schneider et al., 1992).

Both papaya and Arabidopsis contain a single copy of each of the terpene class enzymes. Papaya has more copies of the cytochrome P450 mono-oxygenases than Arabidopsis. There are three copies of KO in papaya compared with only one in Arabidopsis, poplar and rice. These three copies are tandemly repeated within 10 kb of each other and less than 1 kb separates each of the genes. Although all copies contain the *ent*-kaurene oxidase-conserved domain, it is yet unknown if all three continue to function in GA biosynthesis or if subfunctionalization has taken place. Rice contains two functional copies of CPS, but only one copy functions in GA biosynthesis; the second is involved in diterpene phytoalexin biosynthesis (Prisic et al., 2004). The five copies of KAO are located on two different chromosomes. Three of the five copies are located on chromosome 8 and are also tandemly repeated with an average of 1.5 kb of intergenic sequence separating the genes. The tandem duplications of KO and KAO could be the result of retrotransposon activity or unequal crossover.

Unlike the terpene cyclases and P450 mono-oxygenases, papaya has significantly fewer genes in the 2ODD class enzymes. Arabidopsis has almost twice as many copies of each gene family compared with papaya. Papaya and *A. thaliana* are both in the order Brassicales. Their respective families, Car-

icaceae and Brassicaceae, diverged from a common ancestor $\approx$ 72 million years ago (Wikstrom et al., 2001). Since papaya and Arabidopsis diverged, there have been two genomewide duplication events in Arabidopsis and none in papaya (Ermolaeva et al., 2003; Ming et al., 2008), possibly explaining why Arabidopsis has almost twice as many genes in these families as compared with papaya.

During the course of evolution, dioecious plants have arisen continually from hermaphrodite ancestors, and the mechanism controlling sex expression varies between species (Dellaporta and Calderon-Urrea, 1993). Papaya males and hermaphrodites are heterogametic (XY or XY^h) and females are homogametic (XX), similar to dioecious *Silene* that also uses an active Y system for sex determination. Applications of GA₃ do not result in sex conversion in *Silene* (Dellaporta and Calderon-Urrea, 1993). Ghosh and Sen (1975) applied GA₃ to papaya at the seedling stage at 50 ppm and 100 ppm. Although there was a trend toward an increase in male plants in the GA₃ treatment, the results did not differ significantly from the control population. Exogenous applications of ethephon, a chemical that when metabolized is converted into ethylene, also yielded no change in sex expression of papaya (Ming, unpublished results). Similar to *Silene*, sex expression in papaya may not be affected by hormone content.

The application of GA₃ promotes male secondary sexual characteristics in papaya plants but has minimal effect on sex expression and flower development. To further this research, the newly sequenced male-specific region of the Y chromosome will be compared with the hermaphrodite-specific region of the Y^h chromosome and candidate genes will be evaluated to determine function in relation to peduncle elongation. This will yield a better

Fig. 4. Differences in the gibberellin (GA) biosynthetic pathway between *Arabidopsis* and *Carica papaya*. Products are written in black and the enzymes in blue. Each transcript copy for each enzyme is represented by a colored block. The bioactive GAs are highlighted in red and deactivated GAs are highlighted in gray. GGDP, geranylgeranyl diphosphate; CDP, *ent*-copalyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase.

understanding of the gibberellin metabolism pathway and responses in papayas. Elucidating the mechanism controlling peduncle length and flower number in male papaya plants will improve our understanding of how male papaya plants are able to remain competitive against hermaphrodite plants, which are able to self-pollinate.

Literature Cited

Aach, H., H. Bode, D.G. Robinson, and J.E. Graebe. 1997. *ent*-Kaurene synthetase is located in proplastids of meristematic shoot tissues. *Planta* 202:211–219.

Bavrina, T.V., L. Culafic, and M.K. Chailakhyan. 1991. The effect of long day and phytohormones on the flowering and the sex expression of dioecious *Rumex acetosella* L. *Plant. Dokl. Akad. Nauk. SSSR* 317:1510–1514.

Brian, P.W. and H.G. Hemming. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. *Physiol. Plant.* 8:669–681.

Chailakhyan, M.K. 1979. Genetic and hormonal regulation of growth, flowering, and sex expression in plants. *Amer. J. Bot.* 66:717–736.

Chailakhyan, M.K. and V.N. Khryanin. 1978a. Effect of growth regulators and role of roots in sex expression in spinach. *Planta* 142:207–210.

Chailakhyan, M.K. and V.N. Khryanin. 1978b. The influence of growth regulators absorbed by the

root on the sex expression in hemp plants. *Planta* 138:181–184.

Charlesworth, B. and D. Charlesworth. 1978. A model for the evolution of dioecy and gynodioecy. *Amer. Nat.* 112:975–997.

Chen, C., Q. Yu, S. Hou, Y. Li, M. Eustice, R.L. Skelton, O. Veatch, R.E. Herdes, L. Diebold, J. Saw, Y. Feng, W. Qian, L. Bynum, L. Wang, P.H. Moore, R.E. Paull, M. Alam, and R. Ming. 2007. Construction of a sequence-tagged high-density genetic map of papaya for comparative structural and evolutionary genomics in Brassicales. *Genetics* 177:2481–2491.

Davies, P. 1995. *Plant hormones: Physiology, biochemistry & molecular biology*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Dellaporta, S.L. and A. Calderon-Urrea. 1993. Sex determination in flowers. *Plant Cell* 5:1241–1251.

Ermolaeva, M.D., M. Wu, J.A. Eisen, and S. Salzberg. 2003. The age of the *Arabidopsis thaliana* genome duplication. *Plant Mol. Biol.* 51:859–866.

Ghosh, S.P. and S.P. Sen. 1975. The modification of sex expression in papaya (*Carica papaya* L.). *J. Hort. Sci.* 50:91–96.

Hansen, D.J., S.K. Bellman, and R.M. Sacher. 1976. Gibberellic acid controlled sex expression of corn tassels. *Crop Sci.* 16:371–374.

Helliwell, C.A., J.A. Sullivan, R.M. Mould, J.C. Gray, W.J. Peacock, and E.S. Dennis. 2001. A plastid envelope location of *Arabidopsis ent*-kaurene oxidase links the plastid and endo-

plasmic reticulum steps of the gibberellin biosynthesis pathway. *Plant J.* 28:201–208.

Higgins, J.E. and V.S. Holt. 1914. The papaya in Hawaii. USDA, Hawaii Agricultural Experiment Station, BUL 32. Washington, DC.

Hofmeyr, J.D.J. 1938. Genetical studies of *Carica papaya* L. *S. Afr. J. Sci.* 35:300–304.

Khryanin, V.N. 2002. Role of phytohormones in sex differentiation in plants. *Russ. J. Plant Physiol.* 49:545–551.

Lange, A.H. 1961. Factors affecting sex change in the flowers of *Carica papaya* L. *Proc. Amer. Soc. Hort. Sci.* 77:252–264.

Liebman, B. 1992. Fresh fruit: A papaya a day? *Nutrition Action Healthletter*. 10–11.

Liu, Z., P.H. Moore, H. Ma, C.M. Ackerman, M. Ragiba, Q. Yu, H.M. Pearl, M.S. Kim, J.W. Charlton, J.I. Stiles, F.T. Zee, A.H. Paterson, and R. Ming. 2004. A primitive Y chromosome in papaya marks the beginning of sex chromosome evolution. *Nature* 427:348–352.

Ming, R., P.H. Moore, F. Zee, C.A. Abbey, H. Ma, and A.H. Paterson. 2001. Construction and characterization of a papaya BAC library as a foundation for molecular dissection of a tree-fruit genome. *Theor. Appl. Genet.* 102:892–899.

Ming, R., Q. Yu, and P.H. Moore. 2007. Sex determination in papaya. *Semin. Cell Dev. Biol.* 18:401–408.

Ming, R. et al. 2008. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). *Nature* 452:991–996.

Mitchell, W.D. and S.H. Wittwer. 1962. Chemical regulation of flower sex expression and vegetative growth in *Cucumis sativus* L. *Science* 136:880–881.

Mohan Ram, H.Y. and V.S. Jaiswal. 1972. Induction of male flowers on female plants of *Cannabis sativa* by gibberellins and its inhibition by abscisic acid. *Planta* 105:263–266.

Mori, M., T. Nomura, H. Ooka, M. Ishizaka, T. Yokota, K. Sugimoto, K. Okabe, H. Kajiwarra, K. Satoh, K. Yamamoto, H. Hirochika, and S. Kikuchi. 2002. Isolation and characterization of a rice dwarf mutant with a defect in brassinosteroid biosynthesis. *Plant Physiol.* 130:1152–1161.

Peterson, C.E. and L.D. Anghder. 1960. Induction of staminate flowers on gynoecious cucumbers with gibberellin A3. *Science* 131:1673–1674.

Phinney, B.O. 1956. Growth response of single gene dwarf mutants in maize to gibberellic acid. *Proc. Natl. Acad. Sci. USA* 42:185–189.

Pike, L.M. and C.E. Peterson. 1969. Gibberellin A4/A7, for induction of staminate flowers on the gynoecious cucumber. *Euphytica* 18:106–109.

Prisic, S., M. Xu, P.R. Wilderman, and R.J. Peters. 2004. Rice contains two disparate *ent*-copalyl diphosphate synthases with distinct metabolic functions. *Plant Physiol.* 136:4228–4236.

Schneider, G., E. Jensen, C.R. Spray, and B.O. Phinney. 1992. Hydrolysis and reconjugation of gibberellin A20 glucosyl ester by seedlings of *Zea mays* L. *Proc. Natl. Acad. Sci. USA* 89:8045–8048.

Story, W.B. 1953. Genetics of the papaya. *J. Hered.* 44:70–78.

Sun, T.P. and Y. Kamiya. 1994. The *Arabidopsis* GA1 locus encodes the cyclase *ent*-kaurene synthetase A of gibberellin biosynthesis. *Plant Cell* 6:1509–1518.

Sun, T.P. and Y. Kamiya. 1997. Regulation and cellular localization of *ent*-kaurene synthetase. *Physiol. Plant.* 101:701–708.

Tanabe, S., S. Tanabe, M. Ashikari, S. Fujioka, S. Takatsuto, S. Yoshida, M. Yano, A. Yoshimura, H. Kitano, M. Matsuoka, Y. Fujisawa, H. Kato, and Y. Iwasaki. 2005. A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via

- the characterization of a rice dwarf mutant, dwarf11, with reduced seed length. *Plant Cell* 17:776–790.
- Varbanova, M., S. Yamaguchi, Y. Yang, K. McKelvey, A. Hanada, R. Borochov, F. Yu, Y. Jikumaru, J. Ross, D. Cortes, C.J. Ma, J.P. Noel, L. Mander, V. Shulaev, Y. Kamiya, S. Rodermel, D. Weiss, and E. Pichersky. 2007. Methylation of gibberellins by *Arabidopsis* GAMT1 and GAMT2. *Plant Cell* 19:32–45.
- Wang, J., J.K. Na, Q. Yu, A.R. Gschwend, J. Han, F. Zeng, R. Aryal, R. VanBuren, J.E. Murray, W. Zhang, R. Navajas-Pérez, F.A. Feltus, C. Lemke, E.J. Tong, C. Chen, C.M. Wai, R. Singh, M.L. Wang, X.J. Min, M. Alam, D. Charlesworth, P.H. Moore, J. Jiang, A.H. Paterson, and R. Ming. 2012. Sequencing papaya X and Yh chromosomes reveals molecular basis of incipient sex chromosome evolution. *Proc. Natl. Acad. Sci. USA* 109:13710–13715.
- Wikstrom, N., V. Savolainen, and M.W. Chase. 2001. Evolution of the angiosperms: Calibrating the family tree. *Proc. R. Soc. Lond. B Biol. Sci.* 268:2211–2220.
- Yu, Q., R. Navajas-Pérez, E. Tong, J. Robertson, P.H. Moore, A.H. Paterson, and R. Ming. 2008. Recent origin of dioecious and gynodioecious Y chromosomes in papaya. *Trop. Plant Biol.* 1:49–57.
- Zhu, Y., T. Nomura, Y. Xu, Y. Zhang, Y. Peng, B. Mao, A. Hanada, H. Zhou, R. Wang, P. Li, X. Zhu, L.N. Mander, Y. Kamiya, S. Yamaguchi, and Z. He. 2006. ELONGATED UPPERMOST INTERNODE encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. *Plant Cell* 18:442–456.