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Functional genomics of nectar production in the Brassicaceae☆

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ABSTRACT

Nectar is a reward commonly offered by plants to attract potential pollinators, thereby ensuring outcrossing and efficient pollination. Until recently, little research has focused on the molecular components of nectar synthesis, and only a handful of genes have been shown to have a direct effect on nectary function. Recent transcriptomic data have made it possible to identify nectary-related candidate genes and further investigate their potential roles in the synthesis and secretion of nectar. Here we review the current state of research and address how our work aims to close gaps in knowledge relating to the process of nectar production. Using Brassicaceae species as models, we discuss the utilization of molecular and genomic tools available (i.e., sequenced genomes, T-DNA and TILLING mutants, sugar concentration assays, and metabolomics) to gain insight on the complex mechanisms of nectar secretion. Examples of preliminary data from this research are provided, and an online database (www.nectarygenomics.org) housing this data is introduced.

Introduction

Floral nectar is an aqueous solution secreted by flowers as a food source reward for pollinator visitation. This solution plays a vital role in pollinator attraction and in turn may increase fecundity and genetic diversity within a species. Studies have shown a strong correlation to nectar constituents, i.e., quality, which can vary between species, and pollinator preference (Baker and Baker, 1973a; Stuurman et al., 2004). Since plant–pollinator interactions serve highly important biological functions, and are influenced by the secreted reward of nectar, the lack of data on the molecular mechanisms underlying nectar production and secretion is surprising. Aside from knowledge of nectar composition and nectary structure there are little data present on nectary development or the synthesis or secretion of nectar. To this date only three genes have been described to be involved in nectary development: *CRABS CLAW* (*CRC*), *BLADE-ON-PETIOLE* (*BOP*) *1* and *BLADE-ON-PETIOLE 2* (Bowman and Smyth, 1999; McKim et al., 2008). Further, a limited number of genes have been reported to influence nectar production or secretion. For example, Ruhlmann et al. (2010) reported that *CELL WALL INVERTASE 4* is required for nectar production in *Arabidopsis thaliana*; whereas, in tobacco, the transcription factor MYB305 was found to regulate nectary-specific gene expression (Liu et al., 2009). Other current thoughts on the mechanisms and regulation of nectar production have been thoroughly reviewed recently (Heil, 2011; Kram and Carter, 2009).

Our research, along with others, is attempting to elucidate the molecular mechanisms involved in nectar production and secretion in the Brassicaceae. Within our study family, the primary species being worked with are: *Arabidopsis thaliana*, *Brassica napus*, *Brassica oleracea* and *Brassica rapa*. *Arabidopsis* as a model organism provides many genetic tools to examine gene function, but due to its small flowers and even smaller nectaries (~100 μm across), studying proper nectary function can be difficult. Conversely, the *Brassica* sp. have larger flowers and nectaries, but the applicable molecular tools available on *Brassica* are limited compared to those of *Arabidopsis* (explained further in this article).

Nectary structure

Brassicaceae nectaries (and those of members from other families) are composed of three key cell types, (1) epidermal tissue, (2) parenchyma tissue, (3) vascular tissue. The vascular tissue can be composed of both phloem and xylem, e.g., in *Capparis retusa*/Capparidaceae (Di Sapio et al., 2001), or have only phloem,

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as seen in *Arabidopsis* (Baum et al., 2001) and *B. napus* (Davis et al., 1986). In other families, nectary vascularization is missing completely (e.g., *Swietenia*/Meliaceae, petaline nectaries: Paiva, 2011; *Boswellia*/Burseraceae, nectariferous ring: Giuliani et al., 2012). Along with variation in vasculature, how the nectar is released through the epidermis differs from species to species as well, some examples are cuticle microchannels (Davis et al., 1988; Delgado et al., 2011), cuticle disruption (Fiueiredo and Pais, 1992) and stomatal control (e.g., Giuliani et al., 2012). Also the epidermal tissues of *Arabidopsis* and *B. rapa* contain stomatal openings to facilitate nectar secretion (Baum et al., 2001; Davis et al., 1986).

Brassicaceae floral nectaries are often bilobed in shape, and located at the base of the flower behind the sepals. Following this pattern of location, there are four nectary types based on number and distribution of the organs, (1) annular, one continuous ring-shaped nectary surrounding the base of the flower, (2) two-nectary type, two opposing nectaries at the base of the flower, (3) four-nectary type, made up of two pairs of nectaries classified as lateral and median, (4) eight-nectary type, two pairs of lateral and two pairs of median nectaries (Davis et al., 1996, 1998). Our study organisms *Arabidopsis*, *B. napus*, *B. oleracea*, and *B. rapa* have a four-nectary type morphology. The reason for the differentiation between nectary organs, lateral and median, is due to their differing structural characteristics and level of function. Lateral nectaries are often bilobed but can be seen as a single lobed organ; these nectaries also have an appreciable amount of sieve tube elements. Whereas *Arabidopsis* median nectaries are almost always found bilobed with fewer sieve tube elements from the vasculature (Davis, in Bowman, 1994). Further differentiating these nectary pairs is their respective level of nectar production and secretion. *Arabidopsis* lateral nectaries can be responsible for up to 96–100% of total nectar produced by the plant, and the remaining 0–4% are secreted by median nectaries (Davis et al., 1998). It is suggested that the cause of this drastic variation in activity levels is due to the increased amount of vascular sieve tube elements in the lateral nectaries (Davis et al., 1986).

Nectar composition

Nectar is far from a pure sugar water solution; functioning to both attract effective pollinators and deter unwanted "nectar robbers," the nectar composition of a specific plant species is uniquely adapted to the needs and preferences of its desired pollinators (Baker and Baker, 1975). In addition to the main carbohydrate components of sucrose, glucose, and fructose, floral nectars also contain varying amounts of amino acids (Baker and Baker, 1983; Carter et al., 2006), nectar proteins or 'nectarins' (Carter and Thornburg, 2000, 2004), lipids and fatty acids (Baker and Baker, 1975; Kram et al., 2008), phenolics (Baker and Baker, 1983), alkaloids (Adler et al., 2006; Baker and Baker, 1975), organic acids (Baker and Baker, 1975), terpenoids (Raguso, 2004), flavonoids (Truchado et al., 2009), and minerals (Varassin et al., 2001). Functionally, these non-carbohydrate components fulfill a wide range of purposes, serving to increase nutrition and desirability for wanted pollinators (Baker and Baker, 1973b; Mevi-Schutz and Erhardt, 2003), aid in defense against herbivory (Adler et al., 2006), inhibit microbial growth (Carter et al., 2007; Carter and Thornburg, 2004), protect against pathogen infection (Sasu et al., 2010), and deter unwanted nectar thieves (Adler, 2000).

Nectar sugar concentration varies highly among angiosperms, ranging anywhere from 8 to 80% (w/w): Baker and Baker (1983). Our main model system, *Arabidopsis thaliana* ecotype Columbia, displays a nectar hexose (glucose and fructose)-to-sucrose ratio of 33:1, a striking contrast to phloem sugar which is mostly sucrose (Davis et al., 1998), suggesting that processing occurs to modify phloem sap into secreted nectar.

Regulation of nectar production

Few studies have investigated hormonal involvement with floral nectar production. Recent microarray data suggest that hormones play an important role in the development of nectaries, as well as in nectar synthesis and the regulation of nectar secretion (Hampton et al., 2010; Kram et al., 2009). Many genes involved with hormonal regulation, transport, and response were found to be differentially expressed in *Arabidopsis* and *Brassica rapa* nectary tissue. A recent study on *Brassica napus* revealed that jasmonic acid (JA) levels within the flower peak just before nectar secretion, and exogenous application of JA increased the rate nectar was produced (Radhika et al., 2010). Auxin has also been implicated as having a role in nectar production; DR5::GUS staining demonstrated that auxin responsive genes are active in the nectary just prior to pollen release and continued to be active throughout flower maturity (Aloni et al., 2006).

Tools for probing Brassicaceae nectary biology

Both *Arabidopsis* and *Brassica rapa* now have completely sequenced genomes (The *Arabidopsis* Genome Initiative, 2000; Wang et al., 2011) and a number of associated genomic and genetic resources. For example, mutants are readily available for a majority of *Arabidopsis* genes (e.g., Alonso et al., 2003; www.arabidopsis.org), along with a wealth of transcriptomic data under multiple developmental and treatment conditions (e.g., Schmid et al., 2005). While not as developed, tools for functional genomics in *Brassica* sp. are increasingly available, too [e.g., Brassica Database (BRAD); http://brassicadb.org/brad/]. These resources allow the rapid identification of orthologs and synteny between the *Arabidopsis* and *Brassica* sp. genomes. Some additional tools and methods available for specifically investigating nectary function in the Brassicaceae are described below.

Nectarygenomics.org

The aim of our group is to understand the molecular mechanisms behind nectary structure and function in the Brassicaceae. Cumulatively, this project has generated a large amount of genomic and phenomic data. To facilitate public dissemination of this data we have created the website *nectarygenomics.org* and associated database. Some of the highlights of this resource are summarized below.

Expression and metabolomics data

The entirety of our work is predicated on the identification of a large suite of nectary-enriched genes in *Arabidopsis* (Kram et al., 2009) and *B. rapa* (Hampton et al., 2010). Gene expression in wild-type *Arabidopsis* nectaries can be downloaded as a complete dataset under 'DataSet Download', or one can use the 'WildType Microarray Data' tool to select tissues of interest and fold cut-offs. The latter tool allows users to view the data in the browser, or to download the data as a file. Additional transcriptomic data (microarray and RNA-seq) investigating expression in mutant nectaries is available for download under the 'DataSet Download' link.

A detailed analysis of wild-type *Arabidopsis* and *Brassica* sp. nectar composition has been conducted as described below. Moreover, compositional comparisons between wild-type and mutant nectars have been performed. Metabolomics data can be downloaded via

the 'DataSet Download' link; however, work is underway to develop a viewer similar to the one available for expression analyses.

Target genes, mutant lines and phenotypes

Analysis of wild-type nectaries identified over 200 genes expressed 3-fold higher in nectaries than in all other tissues examined (e.g., petal, pollen, leaf, root, stigma, etc.). These candidate genes are now being examined for their roles in nectary function. To date, we have investigated the functions of the 90 candidate genes in *Arabidopsis* listed under 'Target Genes' on the *nectarygenomics.org* website. The page for each gene contains a short description of its known or putative function, its expression patterns (as observed by microarray and/or RT-PCR), and the mutant lines that are being used to examine the biological function of each gene. For closer inspection of the data compiled there the reader is referred to the mentioned website.

The vast majority of mutant lines under investigation are T-DNA mutants (Alonso et al., 2003) readily available through the *Arabidopsis* Biological Resource Center at The Ohio State University in Columbus, Ohio, U.S.A. The list of mutants is available under the 'Mutants' link and individual mutants are also associated with each gene. The phenotype of each mutant is indicated on each mutant's page, and specific phenotypes (e.g., increase/decrease in nectar production) can be searched for under the 'Browse by Category' link.

The development of *nectarygenomics.org* is an ongoing effort. Planned enhancements and updates to *nectarygenomics.org* include the addition of:

- protocols used in mutant isolation and phenotypic analysis;
- SEM and confocal images of mutant and wild-type nectaries;
- Bacterial stocks and seed lines available for distribution;
- DNA constructs available for use in studying nectary and candidate gene function.

Modulation of candidate gene expression in nectaries

pPMK1

Some candidate genes being examined are not exclusively expressed in nectary tissue, and knockout mutants can be lethal or lead to pleiotropic phenotypes, thus making conclusions regarding their direct roles in nectar production impossible. For example, the candidate gene *SUCROSE-PHOSPHATE SYNTHASE 2F* (*SPS2F*, AT5G11110) is highly expressed in mature nectaries (both median and lateral) and at lower levels in many other tissues (Kram et al., 2009). T-DNA knock outs of *SPS2F* produce significantly less nectar (~50% decrease) compared to wild-type; however, mutant plants are also smaller and spindly (i.e., mutants have a pleiotropic phenotype; Gorder et al., in preparation). Thus, it is unknown if *SPS2F* plays a direct role in nectar production.

To address the problems of pleiotropic phenotypes associated with knockout mutants, we have created a plant transformation vector, pPMK1, containing the strong nectary-specific *SWEET9* (At2g39060) promoter. *SWEET9* is one of the most highly expressed genes within nectary tissues (Kram et al., 2009). Specifically, 1.44 kb of the *SWEET9* promoter was cloned into XhoI and BamHI sites of the promoterless vector pORE-O4 (Coutu et al., 2007), which confers kanamycin resistance to transformed plants (Fig. 1A). We have successfully used this vector to drive the expression of artificial microRNAs (amiRNAs) and full length cDNA and genomic fragments. This approach allows the targeted alteration of candidate gene expression in nectaries. One example of pPMK1 being used to overexpress a candidate gene in nectariferous tissue is shown in Fig. 1B.

Brassica rapa TILLING mutants via RevGenUK

One useful tool for functional genomics of nectaries is Targeting Induced Local Lesions IN Genomes (TILLING). TILLING is a powerful reverse genetics tool used to identify random point mutations within a particular gene of interest in a plant's genome (McCallum et al., 2000). Chemical treatments such as ethyl methane sulfonate (EMS) to seeds or pollen can lead to random GC → AT mutations, via alkylation of the guanine's O^6 position (Ashburner, 1989). Due to the GC → AT type of mutation, nonsense mutations are highly likely, therefore producing truncated protein products. Stephenson et al. (2010) have presented methods to generate TILLING mutants in *Brassica rapa* (R-o-18), which has increasing significance due to the sequenced genome of *B. rapa* Chiffu-401. Cumulatively, the fully sequenced genome of Chiffu-401 and mutageneized pool of R-o-18 *B. rapa* are tools vital to the study of gene function in *B. rapa*. The John Innes Center-based RevGenUK (http://revgenuk.jic.ac.uk/) has begun screening services to identify TILLING mutants in the R-o-18 background based on user-defined sequences. TILLING mutants for two orthologs of *Arabidopsis* genes known to be involved in nectar production are shown in Table 1 as an example. One obstacle of the TILLING method is the random and multiple point mutations from the EMS treatment; mutations are generated throughout the genome, not only in the target gene. To clean up the unwanted

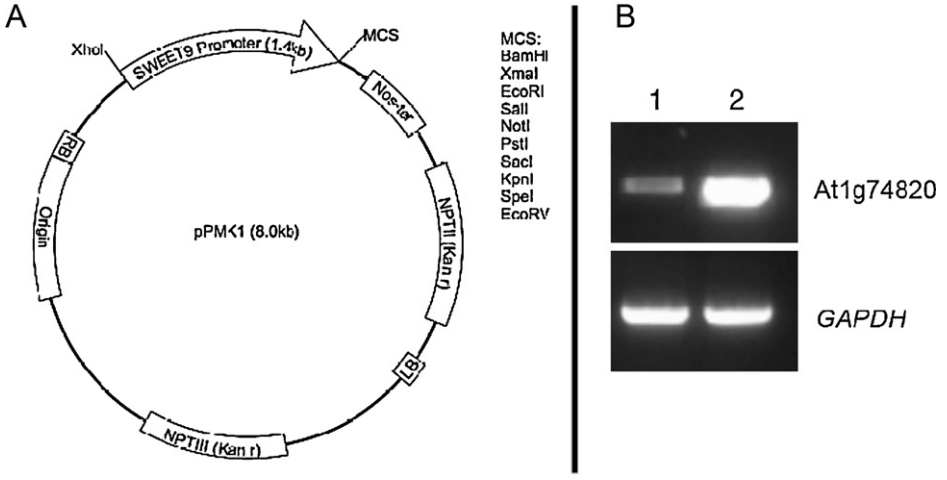

Fig. 1. Map of pPMK1, a transformation vector containing a nectary-specific promoter. (A) Map of pPMK1 containing 1.44 kb of the strong nectary-specific *SWEET9* promoter ligated into the plasmid vector pORE-O4. (B) RT PCR expression analysis of the lowly expressed candidate gene At1g74820 in the flowers of wild-type, lane 1, and transformed plants with the coding region of At1g74820 under control of the *SWEET9* promoter, lane 2.

Table 1
Non-silent *B. rapa* TILLING mutants from *RevGen*UK.

Gene	Plant name[a]	Mutation[a]
BrPIN6 (Bra015694)	JI32022-A	R279W
	JI33068-A	G126R
	JI31975-B	E127K
	JI31792-A	A156T
	JI32852-A	R162Q
	JI31981-A	P166L
	JI31967-B	G167S
	JI33067-A	A170T
	JI32362-A	E194K
	JI32960-B	T195A
	JI32901-A	G201D
	JI32454-B	V214I
	JI31606-B	P215L
	JI32743-B	S239F
	JI32757-B	G251E
	JI31758-B	R162 > stop
	JI32506-A	432G > A (splice site)
BrCWINV4 (Bra017257)	JI32925-A	G82D
	JI32654-A	T88I
	JI32252-A	G111R
	JI32970-A	G132S
	JI32527-B	S171F
	JI32040-A	G226E
	JI31928-B	A232V
	JI32694-B	G241E
	JI32807-A	R295K
	JI31935-B	W159 > stop

[a] Mutants for the orthologous genes in *Arabidopsis* display altered nectary phenotypes.

background point mutations multiple backcrosses to the parent line are required.

Phenomics of nectar and nectaries

Naturally, obtaining mutants for candidate genes is only the first step in performing functional genomics of nectar production. Several suggested methods and recent developments in phenotyping nectaries and nectar are described below. Other methods, particularly for imaging nectaries, were previously described (Kram and Carter, 2009).

Nectar collection

The vast majority (>99%) of *Arabidopsis thaliana* Col-0 nectar is produced by the lateral nectaries (Davis et al., 1998). Thus, our *Arabidopsis* nectar samples are prepared by carefully pulling back sepals covering the lateral nectaries and touching the nectar droplet with small, uniform triangular wicks cut from Whatman No. 1 filter paper under a dissecting microscope. For standardization purposes, nectar from a set number of flowers (e.g., usually 20 flowers) is collected on one wick prior to being placed in 100 μl of nuclease free water for solute elution, making one replicate. For metabolite profiling via GCGC–MS (described below) a total of six replicates are made for Col-0 and each mutant line used. For routine sugar analyses (also described below), *Arabidopsis* nectar samples are prepared using the same nectar collection procedure as for GCGC–MS analysis, but nectar is collected from 10 flowers per wick and then placed in 500 μl of sterile water to elute the solutes. A similar procedure was previously described for nectar collection from *Arabidopsis* flowers with cut pedicels cultured in sugar solutions (Davis et al., 1998); however, we have found culturing is not necessary to obtain enough sample material for metabolite analyses from flowers grown in situ. Nonetheless, culturing *Arabidopsis* flowers in sugar solutions containing specific treatments (e.g., hormones) is useful for examining regulatory mechanisms of nectar production (Fekete, 2011).

Brassica sp. nectar samples are readily collected with 2 μL micropipettes (Drummond Scientific, cat. no. 1-000-0020), with the fluid then expelled into microcentrifuge tubes. Significantly, one can collect 100 μL of nectar from wild-type flowers within a day. In our work, nectar samples are clarified by centrifugation and always stored at −80 °C until analysis to avoid sample degradation.

One significant consideration when performing nectar collection is timing, both developmental and circadian. This is because nectar production in *Brassica* sp. undergoes circadian oscillations. For example, nectar production was found to be maximal between 4 and 8 h after dawn (h.a.d.) in *Brassica napus* (Búrquez and Corbet, 1991). We have observed a nearly identical pattern of nectar production in *Arabidopsis* (Bender and Carter, unpublished). Thus, when making comparisons, e.g., wild-type vs. mutant, we only collect nectar from flowers 4–8 h.a.d., and alternate between collection from mutant and wild-type plants if more than one biological replicate is being collected. Similarly, samples are only collected from Stage 14–15 flowers on plants of the same age that have been grown side-by-side under the exact same light, water and fertilizer regime.

Sugar assays as a measure of nectar production

Arabidopsis nectar was previously reported to be hexose dominant. Indeed, little or no sucrose was observed, and glucose and fructose was found in close to a 1:1 ratio (Davis et al., 1998). As such, measurement of glucose alone can be used as a proxy for total nectar production. In our studies, glucose concentration of *Arabidopsis* nectar samples is analyzed according to previously described methods utilizing glucose oxidase and Ampliflu™ Red (Bethke and Busse, 2008; Ruhlmann et al., 2010). Briefly, 75 μl of eluted nectar sample (nectar from 10 flowers collected on a paper wick placed in 500 μl diH_2O) is combined with 25 μl of Amp-Red enzyme mix and incubated for 30 min in the dark at room temperature. Sample absorbance is measured at 560 nm, which is directly proportional to glucose concentration. The Amp-Red enzyme mix is prepared by mixing 862.5 μl 150 mM $NaPO_4$ (pH 7.4), 1 unit of horseradish peroxidase (Sigma cat. no. P8250), 10 units of glucose oxidase (Sigma cat. no. 49180), and 100 μl of 10 mM Ampliflu™ Red in DMSO (Sigma cat. no. 90101) to a volume of 2.6 ml. If desired, standard curves can be generated to provide an estimate of total glucose (Bethke and Busse, 2008); however, relative changes in nectar production can be observed by utilizing the absorbance of wild-type and mutant samples collected side-by-side as described above. One example of the results from an assay comparing wild-type *Arabidopsis* and a mutant line are presented in Fig. 2. The same approach for nectar sugar analysis is also applicable for the hexose-dominant *Brassica* nectars.

Metabolomic analyses

While sugars are the predominant solutes found in nectars, other minor constituents play important roles in plant–animal interactions (e.g., Adler, 2000; Adler et al., 2006; Baker and Baker, 1973b, 1975; Carter et al., 2006). The W.M. Keck Metabolomics Research Laboratory at Iowa State University has developed methods for in-depth metabolomic analyses of nectars using a novel GCGC–MS technique. This new instrumentation has the ability to separate metabolites by conducting gas chromatography (GC) in tandem. Namely, the instrument separates the metabolites by conducting sequential separation of the metabolites through two tandem GC columns. The second column can be used to store undesired peaks or compounds that need further separation. The analytes that are separated by the two tandem GC columns are detected by a mass-spectrometer (MS), which also provides a

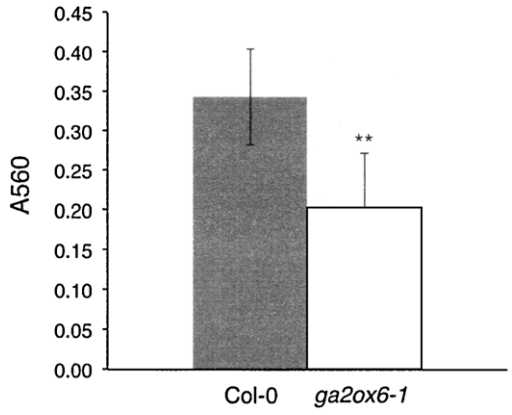

Fig. 2. Example of *Arabidopsis* nectar sugar assay. There is a clear change in total nectar glucose in the *ga2ox6-1* knock-out mutant (SALK_044189C for At1g02400) compared to wild-type Arabidopsis (Col-0). $^{**}p = 0.004$.

means of identifying the individual metabolites. Because analytes are separated by a tandem arrangement of GC columns, this technology, analogous to 2-dimensional PAGE for proteomics analysis, has the power of separating highly complex mixtures of metabolites. The extraction method that we applied separates the metabolites according to the polarity nature. As a result, this extraction protocol generates both polar and nonpolar fractions. Both of these fractions are evaluated through this combined GCGC–MS method to provide information on nectar metabolites. In addition to metabolite identification, the relative amount and total concentration of each metabolite can be determined. Pooled nectar samples from wild-type and mutant *Arabidopsis* and *Brassica* plants, as described above, have been used for nectar compositional analyses. For example, nectar collected from all six *Brassica* sp. (*B. rapa*, *oleracea*, *juncea*, *napus*, *nigra* and *carinata*) has been analyzed, and nearly 40 metabolites have been identified. They include sugars, amino acids, organic acids, mineral acids, fatty acids, hydrocarbons, and secondary compounds. As one example, the metabolite profile of *B. rapa* nectar is shown in Table 2. Significantly, PCA analysis indicates that nectars from the *Brassica* species can be discriminated from one another via these methods (Perera et al., in preparation).

Table 2
Mean concentration of solutes in *B. rapa* nectar as detected by GCGC–MS.

Metabolite	Conc. (*M*)	S.D. (*M*)
Glucose	8.042	0.539
Ribose	7.216	0.479
Fructose	0.850	0.158
Gluconic acid	0.665	0.269
Xylulose	0.565	0.142
Turanose	0.474	0.064
Arabinonic acid	0.276	0.081
Acrylic acid	0.249	0.106
Hexanedioic acid	0.183	0.070
Talose	0.172	4.7×10^{-3}
Hexadecanoic acid	0.156	0.031
Erythro-pentonic acid	0.142	0.057
Octadecanoic acid	0.119	0.125
Erythrotetrofuranose	8.50×10^{-2}	3.37×10^{-2}
Threonic acid	3.92×10^{-2}	3.82×10^{-3}
Scopolin	3.84×10^{-2}	8.78×10^{-3}
Butane	3.53×10^{-2}	1.58×10^{-2}
Galactose	2.95×10^{-2}	3.94×10^{-3}
Myo-inositol	2.78×10^{-2}	1.44×10^{-3}
Propanoic acid	1.77×10^{-2}	3.32×10^{-3}
Myristic acid	1.55×10^{-2}	2.21×10^{-3}
Eicosanoic acid	8.43×10^{-03}	8.10×10^{-3}
Phenol	7.38×10^{-03}	6.79×10^{-3}
Mannose	6.27×10^{-03}	1.71×10^{-3}
Inositol	4.07×10^{-03}	1.25×10^{-3}
Arabinose	2.06×10^{-03}	1.10×10^{-3}
Tetradecanoic acid	1.63×10^{-03}	1.77×10^{-3}
Glycine	1.21×10^{-03}	9.42×10^{-4}
Arabino-hexonic acid	7.67×10^{-04}	7.83×10^{-4}
Erythro-pentofuranose	4.60×10^{-04}	3.77×10^{-4}
Xylitol	4.57×10^{-04}	4.18×10^{-4}
Ribonic acid	3.90×10^{-04}	2.03×10^{-4}
Phosphoric acid	2.60×10^{-04}	1.82×10^{-4}
Arabinonic acid	1.96×10^{-04}	1.72×10^{-4}
Propanedioic acid	1.89×10^{-04}	7.81×10^{-5}

Concluding remarks

Here we have discussed the current status of existing and still lacking knowledge pertaining to the molecular mechanisms of nectary function, and how a number of techniques can be employed to further address unanswered questions. In order to form a complete understanding of the complex process of nectar production, future work needs to move beyond single gene investigations and begin to examine the connections between the underlying genetic, cellular and metabolic mechanisms of nectary function. Special attention should be focused on hormonal influences and associated signaling pathways, and how these factors impact nectar production and secretion. Of course, ultimately, true biological function will rely on determining candidate gene impacts on nectar quality, pollinator visitation, and overall fecundity.

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