Secretory Phospholipases A2 in Plants

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Secreted phospholipases (sPLA2s) in plants are a growing group of enzymes that catalyze the hydrolysis of sn-2 glycerophospholipids to lysophospholipids and free fatty acids. Until today, around only 20 sPLA2s were reported from plants. This review discusses the newly acquired information on plant sPLA2s including molecular, biochemical, catalytic, and functional aspects. The comparative analysis also includes phylogenetic, evolutionary, and tridimensional structure. The observations with emphasis in Glycine max sPLA2 are compared with the available data reported for all plants sPLA2s and with those described for animals (mainly from pancreatic juice and venoms sources).

Keywords: secretory phospholipase A2, interfacial catalysis, auxin, Glycine max, phospholipase–membrane interaction

INTRODUCTION

For more than a century experiments were performed with sPLA2s enzymes, being used as lipid model enzymology and as paradigms for the formalism of interfacial catalysis (Dennis et al., 2011). The phospholipase A2 (PLA2, EC 3.1.1.4) superfamily is a broad and growing group of enzymes that stereo specifically catalyzes the cleavage at the sn-2 acyl ester bond from diacyl-phospholipid liberating lysophospholipid and free fatty acid. In plants, secreted PLA2 (sPLA2) represents one type of phospholipase A2 whose lipid products mediate a variety of cellular processes, including growth, development, defense, and stress responses (Stahl et al., 1998, 1999; Kim et al., 1999; Lee et al., 2003; Ryu, 2004; Mansfeld, 2009; Chen et al., 2011). Although numerous sPLA2 genes have been identified in plants, little is known about these enzymes in opposition to their insect, animal or human counterparts (Burke and Dennis, 2009). sPLA2 is best known from mammals where several sPLA2s have been identified in the last 25 years (Murakami et al., 2011). Moreover, many sPLA2s were found in sources as venoms from snakes, scorpions, bee, etc.; from microorganisms as bacteria and yeasts, as components of pancreatic juices, where it occurs abundantly and has a digestive role; arthritic synovial fluid; and in many different mammalian tissues (Valentin et al., 1999; Schaloske and Dennis, 2006; Burke and Dennis, 2009; Murakami et al., 2010, 2011). Additionally, for the first time, we have recently described the interfacial properties of purified recombinant sPLA2s from Streptomyces violaceoruber (Yunes Quartino et al., 2015) and from Glycine max (Mariani et al., 2012, 2015b), i.e., the optimal surface lipid packing conditions (interfacial quality) in which a sPLA2 can hydrolyze phospholipid in an organized membrane. This point, no less important for interfacial enzymes, was also addressed comparatively in the present review.

Glycine max (Soybean), in addition to being one of the most widely used oil crop grain in the world, possesses valuable contributions to health due to its high nutritional level. Lipids, proteins and other valuable bioactive components such as: phospholipids (known as lecithin), hormones,
and antioxidants are present in soybean (Messina, 1999; Choi and Rhee, 2006). The industrial use of sPLA₂s from animal pancreas extracts and microbes, especially in food production, has a long tradition (Guo et al., 2005; De Maria et al., 2007). One of the targets in the future may be the utilization of sPLA₂ from plants for enzymatic processing to stereospecifically obtain lysoderivatives. This alternative has been recently recognized to satisfy food regulation requirements such as Kosher and Halal (Havinga, 2010). However, no sPLA₂s from plants have now been yet available for industrial application (Mansfeld, 2009).

Secretd PLA₂₅₈ are low MW calcium dependent enzymes (12–18 kDa) (Schaloske and Dennis, 2006). From a perusal revision of sequence data, almost all sPLA₂₅₈ from plants and animals contain a signal sequence. So, in the general secretion way after removal of the N-terminal signal peptide in the endoplasmic reticulum (ER), they are secreted into the extracellular space in a either mature or pre-protein form (Fujikawa et al., 2005; Lee et al., 2005; Mansfeld et al., 2006). Although sPLA₂₅₈ are recognized to be secreted proteins, a few of them were reported to act intracellularly prior or during secretion (Mounier et al., 2004; Shridas and Webb, 2014). Until now, the pre-protein form would be exclusive for animals (see Table 1).

Important common features shared for all sPLA₂s are the presence of: (i) one HIS residue at the catalytic domain for nuclophilic attack at the sn-2 acyl ester bond of the glycerol backbone, (ii) requisite of calcium for full activity (HIS-ASP/X (where X may be either HIS, SER, or ASP) induced catalytic mechanism is by checking if the activity is chemically canceled by the alkylation of HIS localized in the catalytic triad (Stahl et al., 1998). They also showed high resistance to organic solvents, acidic conditions and high temperatures (they are even more resistant in the presence of Ca²⁺). A common procedure to confirm the catalytic mechanism is by checking if the activity is chemically canceled by the alkylation of HIS localized in the catalytic triad HIS/ASP/X (where X may be either HIS, SER, or ASP) induced by p-bromophenacyl bromide (BPB) (Minchietti et al., 2008). A resume of the general characteristics comparing animals from plants sPLA₂ is shown in Table 1.

Fatty acids produced by the hydrolysis carried out by sPLA₂s, such as oleic (1:18) or arachidonic (4:20) acid, are sources of energy reserve. Furthermore, arachidonic acid can function as intracellular second messenger or as precursor of eicosanoids inflammation mediators, if it is the extracellular product of the reaction catalyzed by secreted phospholipase as occurs for human synovial fluid (Baynes and Marek, 2004). The other product of the action of sPLA₂, the lysophospholipid is important in cell signaling and remodeling or membrane perturbations (Khan et al., 1995). In contrast, in plants the jasmonic acid and its related compounds are important hormones involved in plant defense reaction against microbial pathogens, herbivores and UV light damaging as well as senescence mechno-transduction (Schaller, 2001).

In the past years, significant advances have been made toward understanding the role of these enzymes in normal cellular and tissue homeostasis or function particularly in mammals (Rhee and Bae, 1997; Assmann and Shimazaki, 1999; Williams, 1999; Liscovitch et al., 2000; Murakami et al., 2015) but, the more recent data reported for plant sPLA₂₅₈ are rather scarce. Therefore, this review focuses on recently acquired information on all sPLA₂s from plants reported until now with emphasis in GmsPLA₂₅₈ identified in G. max (soybean), comparing them with the more relevant published data for several sPLA₂s obtained from different sources. A comparative description with respect to the sequence characterization, biochemical, molecular, and functional aspects of sPLA₂₅₈ enzymes was done.

### TABLE 1 | General characteristics presented by calcium dependent sPLA₂s from animals to plants.

| Properties/characteristics | Animals | Plants |
|----------------------------|---------|--------|
| Intracellular second messenger | PL → arachidonic acid → prostaglandins and leukotrienes | PL → linoleic acid → jasmonic acid |
| Main metabolic pathway | Eicosanoid pathway | Octadecanoid pathway |
| Secreted aszymogen | Some | NR |
| Catalytic triad | ASP/HIS/ASP (K = ASN or SER or HIS) | ASP/HIS/X |
| MW (kDa) | 12–18 | ~14 |
| Cysteines | 8–14 | 12 |
| Disulphide bridges | 4–7 | 6 |
| Calcium requirement | mM² | μM-MM³ |

PL, phospholipid; NR, no reported. *Minimum of required Ca²⁺ concentration for full activity. **Regarding to the general mM requirement for reported sPLA₂s from animal source, it has been described one exception for a sPLA₂ isolated from venom of the marine snail Conus magus (McIntosh et al., 1995). This exception for sPLA₂ was also remarked by Six and Dennis (2000).**

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In comparison with the animal sPLA₂, the knowledge generated for sPLA₂ from plants is still limited, even though when recombinant enzymes from plants have been recently expressed in *Escherichia coli* and yeast and characterized. Some studies about enzyme activities have been reported in more or less
TABLE 2 | sPLA$_2$s from plants, accession numbers, N-terminus characteristics and purification/recombinant process applied.

| Source          | Name            | Purification | N-terminal$^b$ | Accession number | Group XI | References                      |
|-----------------|-----------------|--------------|---------------|------------------|----------|---------------------------------|
| Arabidopsis     | AtsPLA$_2$-α    | cDNA         | Recombinant   | At2g06925        | B        | Mansfeld and Ulbrich-Hofmann, 2007 |
|                 | AtsPLA$_2$-β    | cDNA         | Mature        | At2g19660        | A        | Lee et al., 2003                |
|                 | AtsPLA$_2$-γ    | cDNA         | Mature        | At4g29460        | A        | Bahn et al., 2003               |
|                 | AtsPLA$_2$-δ    | cDNA         | NR            | At4g9470         | A        | Bahn et al., 2003; Ryu et al., 2005 |
| R. communis     | RsPLA$_2$-α     | cDNA         | Recombinant   | XM002523613      | B$^b$    | Bayon et al., 2015              |
| (castor bean)   | RsPLA$_2$-β     | cDNA         | Recombinant   | XM002514118      | B$^b$    | Bayon et al., 2015              |
| C. sinensis     | CcPLA$_2$-α     | cDNA         | Recombinant   | GU075936         | B$^b$    | Liao and Burns, 2010            |
| (oats)          | CcPLA$_2$-β     | cDNA         | Recombinant   | GU075938         | A$^b$    | Liao and Burns, 2010            |
| D. caryophillus (carnation) | DcPLA$_2$ | cDNA         | NR            | AF064732         | B        | Kim et al., 1999                |
| U. glabra$^b$ (elms) | UgsPLA$_2$ | Fusion Protein | NR            | NR               | NR       | Stahl et al., 1998              |
| G. max          | GmsPLA$_2$-X0A-I| cDNA         | Mature        | BT092274         | A        | Mariani et al., 2012            |
| (soybean)       | GmsPLA$_2$-X0A-II| NR          | NR            | BT094841         | A        | Mariani et al., 2012            |
|                 | GmsPLA$_2$-XIB-I| cDNA         | NR            | BT095220         | B        | Mariani et al., 2012            |
|                 | GmsPLA$_2$-XIB-II| cDNA         | Mature        | BT091171         | B        | Mariani et al., 2015b           |
|                 | GmsPLA$_2$-XIB-III| NR          | NR            | BT099163         | B        | Mariani et al., 2012            |
| L. usitatissimum | LusPLA$_2$-I    | cDNA         | Fusion Protein | KU361324        | B        | Gupta and Dash, 2017; Gupta et al., 2017 |
| (flax)          | LusPLA$_2$-II   | cDNA         | Fusion Protein | KU361325        | A        | Gupta and Dash, 2017; Gupta et al., 2017 |
| P. somniferum (opium) | PsPLA$_2$ | cDNA         | Recombinant   | KU900749         | B        | Jablonicka et al., 2016         |
| O. sativa (rice) | OssPLA$_2$-I    | Seeds        | PPiE          | AJ238116         | A        | Lee et al., 2005                |
|                 | OssPLA$_2$-II   | cDNA         | Mature        | AJ238117         | B        | Stahl et al., 1999; Guy et al., 2009 |
| N. tabacum (tobacco) | Nt1PLA$_2$ | cDNA         | Recombinant   | AB190177         | A        | Fujikawa et al., 2011           |
| L. esculentum (tomato) | LePLA$_2$ | Extract      | PPiE          | AB190178         | B        | Fujikawa et al., 2011           |
| T. durum (durum wheat) | TdSPS PLA$_2$I | cDNA/LE     | PPiE          | JX021445         | A        | Verlotta et al., 2013           |
|                 | TdSPS PLA$_2$II | cDNA/LE     | PPiE          | JX021446         | B        | Verlotta et al., 2013           |
|                 | TdSPS PLA$_2$III| cDNA         | Recombinant   | JX021447         | B        | Verlotta et al., 2013; Verlotta and Trono, 2014 |
| Z. mays (maize) | ZmsPLA$_2$-I   | cDNA/LE     | PPiE          | JX021448         | B        | Verlotta et al., 2013           |

$^b$Mature, without extra amino acids at the N-terminus after heterologous expression. PPiE, when the enzyme was Partially Purified from Extracted from a plant organ (partial purification, less than 90% purity). LE, leaves extract. Recombinant is indicated when, according to the reported data, it is not known if the expression assayed is in mature form or contain any tags in the final purified recombinant form (no clearly indicated in the original paper). NR, not reported. $^c$For sPLA$_2$ from Ulmus glabra (elms) it was assigned as UgsPLA$_2$ since in the original describing paper (Stahl et al., 1998) was named as sPLA$_2$ without initial letters of identification. $^d$Named as ZmsPLA$_2$ in this review.

The first sPLA$_2$ purified to homogeneity, sequenced and characterized from plants, was the sPLA$_2$ from elm seed endosperm (Ulmus glabra) in 1998 (Stahl et al., 1998). Later in 1999, two cDNAs encoding sPLA$_2$ (sPLA$_2$-I and-II) were isolated from shoots of rice (Oryza sativa) and characterized (Stahl et al., 1999). cDNAs full sequences coding for putative sPLA$_2$s were obtained from flowers of carnation (Dianthus caryophyllus) (Kim et al., 1999). These later clones from carnation and rice have not been further characterized to demonstrate that they encode functional enzymes. With progress in genome sequencing projects, more sPLA$_2$s have been identified: in tomato (Lee et al., 2005) and outbreaks of castor bean (Ricinus communis) (Domínguez et al., 2007). Four isoforms of sPLA$_2$ from Arabidopsis thaliana have been also isolated, called AtsPLA$_2$-α, -β, -γ, and -δ (Bahn et al., 2003; Lee et al., 2003, 2005; Mansfeld and Ulbrich-Hofmann, 2007; Seo et al., 2008), which have been expressed (Ryu et al., 2005; Mansfeld and Ulbrich-Hofmann, 2007) two isoforms have been studied in tobacco (Nicotiana tabacum) (Dhondt et al., 2006; Fujikawa et al., 2005, 2011) and orange (Citrus sinensis) (Liao and Burns, 2010). Three cDNA from durum wheat (Triticum durum) were isolated and two of them studied in detail (Verlotta et al., 2013; Verlotta and Trono, 2014). A novel sPLA$_2$ from opium (Papaver somniferum) was purified and characterized (Jablonicka et al., 2016) and two crude preparations (Moreau and Morgan, 1988; Mukherjee, 1990; Minchiotti et al., 2008; Murakami et al., 2011).
sPLA$_2$ from flax (*Linum usitatissimum*) were studied in detail (Gupta and Dash, 2017; Gupta et al., 2017). Moreover, one gene was reported for tomato (*Lycopersicon esculentum*) (Lee et al., 2005) and one gene for maize (*Zea mays*) found in UniProt and mentioned in (Mariani et al., 2012). From our laboratory, five *G. max* phospholipases A$_2$ were reported (Mariani et al., 2012), and two of them (*GmsPLA$_2$-XIA-I* and *-XIB-II*) were cloned, expressed in *E. coli*, further purified from inclusion bodies and the activity was evaluated using organized lipid systems such as mixed micelles and monomolecular films as substrates (Mariani et al., 2015b).

Table 2 summaries the different enzymes found in plants, their origin and source, GenBank accession numbers and the subgroup at which they belong to within the XI group of the PLA$_2$ superfamily.

### RECOMBINANT vs. NATIVE sPLA$_2$S PROTEINS: ROLE OF THE INTACT N-TERMINAL PRESERVATION

Usually the N-terminus region of sPLA$_2$ has an alpha helix domain which forms one wall of the channel through which the hydrophobic substrate entries as reported for groups I and II sPLA$_2$S enzymes (according to Dennis, 1973b). Thus, in the case of pancreatic enzyme (group I), when the zymogen is converted into the active form by removing a short portion of the N-terminus, the remaining N-terminal helix is now able to be involved in the binding interfacial membrane (Scott et al., 1990). This would be affected by the extension of seven amino acids at the N-terminus in the zymogen (pro-enzyme) preventing the binding to lipid interfaces. Crystallographic evidence suggests that the zymogen has a more flexible N-terminus compared to the mature protein (van Deenen, 1971).

The effect of an extra amino acid on the N-terminus of pancreatic sPLA$_2$ can be critical, for example, if it is of hydrophobic nature (van Scharrenburg et al., 1984). This was observed in the pioneering work of deHaas group, showing that the extension of an amino acid (doubling of the terminal ALA of the mature form) caused a decrease in enzyme catalysis to phosphatidylcholine (PC) short chain substrate presented as micelles or when the substrate was arranged as a lipid monolayer (Slotboom et al., 1977). Furthermore, in the case of porcine pancreatic enzyme, an absolute free amino terminal is required (Dijkstra et al., 1984).

In a recent work with a sPLA$_2$ from group II of *Crotalus atrox* venom, the importance of a native N-terminus was also evident. By using chemically modified enzyme the authors concluded that N-terminal region plays a mechanistic role in catalysis and acts as a surface-active component of the complex interfacial catalytic site (Randolph and Heinrikson, 1982). This structural requirement is also found in other sPLA$_2$ expressed in bacteria, such as human sPLA$_2$ from synovial fluid (Marki and Hanulak, 1993). It was observed that, when expressing a sPLA$_2$ in *E. coli*, the initial MET is not removed from the protein that had an ASN at position 1 in the sequence. This is because the bacterial aminopeptidase does not catalyze the removal of the initial MET if it is followed by ASN. The lipolytic activity of this protein was very low relative compared with the expressed correct N-terminus mature form (Othman et al., 1996). Similarly, another study reported that the protein with an extra MET at its N-terminus had the same pH optimum and preferred substrate compared to the one with native end (without MET), but the activity was drastically reduced (Marki and Hanulak, 1993). Bacterial aminopeptidases remove initial MET efficiently when the amino acid in position 2 of the mature sequence is little and without charge (such as ALA, GLY, SER), but fail when the residue is voluminous and charged as ASN (Hirel et al., 1989). Othman et al. (1996) have substituted the ASN by ALA to express the recombinant protein thus allowing the removal of the initial MET by the bacteria and avoiding a subsequent step of chemical or enzymatic cleavage.

A similar observation was made in sPLA$_2$ mutants from Taiwan cobra (*Andersen and Dufton, 1997*). The addition of a MET at the N-terminus generates structural distortions, and it was postulated that affects the active site through hydrogen bonds network. Moreover, an extra MET decreases the activity with respect to the enzymes with native end (Chiu et al., 2008). Some reports suggest that the N-terminal helix of groups I and II sPLA$_2$S acts as a regulatory domain that mediate the interfacial activation (Qin et al., 2005).

The correct design of the heterologous expression of the cloned enzyme is crucial because the recombinant protein must be generated with the correct native N-terminus, without any additional amino acid extension, since any modification or extension of the N-terminus in sPLA$_2$ can severely alter the catalytic properties (van Scharrenburg et al., 1984; Othman et al., 1996). This is also valid for any additional N-terminal tag (such as HIS-Tag, frequently used in molecular biology protocols to express recombinant proteins). Both facts make the recombinant protein act as a zymogen like pre-protein.

In this sense, the sPLA$_2$S obtained from *G. max* were expressed without N-terminal extension (Mariani et al., 2012, 2015b) by using the pHUE vector system that utilizes the ubiquitin fusion technique (Catanzariti et al., 2004), which allows easy purification and high yield of recombinant proteins (see Figure 1). The *E. coli* pHUE vector permits the expression of a particular protein as HIS-tagged ubiquitin fusion. Then, the HIS-tag-ubiquitin-sPLA$_2$ fusion is further processed by the deubiquitylating enzyme used to cleave off the fusion to obtain the protein of interest free of any N-terminal extension (Figure 1).

In the particular case of the mature protein *GmsPLA$_2$-XIB-II*, the LEU amino acid at the N-terminus was mutated to an ALA, to optimize the chance of obtaining the correct refolding as previously recommended (Kohler et al., 2006). In AtsPLA$_2$-α, it was shown that an uncleaved signal peptide of the pre-processed forms produced a significant suppression of activity compared with the corresponding mature protein form (Ryu et al., 2005). Moreover, other sPLA$_2$S from plants were expressed without the signal peptide (Mansfeld et al., 2006;
Guy et al., 2009). In animals, a correct and functional sPLA2 from Bothrops asper was produced without any extra extension at the N-terminus (Yunes Quartino et al., 2012). Using the ubiquitin/deubiquitinate system, in the latter case, it was clearly shown that the recombinant protein had the same interfacial catalytic profile when compared to the native one (Yunes Quartino et al., 2015).

As sPLA2 activity is very sensitive to N-terminus modifications, in Table 2 we include all sPLA2s from plants known until today and the process originally reported to obtain the final protein (either proteins purified from plant extracts, in a mature recombinant form or with an additional tag). It should be noted that not all reported information disclosed the sequence of phospholipase A2 either cloned or purified.

However, similar to its counterpart in animals, sPLA2s from plants have N-terminal signal peptides that were predicted to direct protein secretion into the extracellular or intracellular space (Bahn et al., 2003; Lee et al., 2003). It is noteworthy that some sPLA2s from plants have the sequences KTEL, KFEL, and KLEL at the C-terminal which are similar to the endoplasmic reticulum retention sequences KDEL and HDEL reported for animals (Pagny et al., 2000; Seo et al., 2008) is present in some plant sPLA2, the biochemical significance is still unknown (see Supplementary Figure S1).

### Table 3 | Sequence characteristics of the GmsPLA2s

| Name          | Full-length cDNA (nt) | Open reading frame (ORF) (nt) | Residues of native protein with signal peptide | Residues of mature protein |
|---------------|-----------------------|------------------------------|-----------------------------------------------|---------------------------|
| GmsPLA2-XIA-I | 789                   | 417                          | 138                                          | 114                       |
| GmsPLA2-XIA-II| 875                   | 417                          | 138                                          | 115                       |
| GmsPLA2-XIB-II| 826                   | 474                          | 157                                          | 128                       |
| GmsPLA2-XIB-II| 762                   | 471                          | 156                                          | 128                       |
| GmsPLA2-XIB-III| 821                  | 477                          | 158                                          | 128                       |

*In Supplementary Figure S5 it is shown the complete sequence with the N-terminal region with the signal peptide and the putative theoretical site of cleavage for all sPLA2 reported for plants. Signal peptides for each sequence were determined by using the signalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP-3.0/).*

In animals, a correct and functional sPLA2 from Bothrops asper was produced without any extra extension at the N-terminus (Yunes Quartino et al., 2012). Using the ubiquitin/deubiquitinate system, in the latter case, it was clearly shown that the recombinant protein had the same interfacial catalytic profile when compared to the native one (Yunes Quartino et al., 2015).

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However, similar to its counterpart in animals, sPLA2s from plants have N-terminal signal peptides that were predicted to direct protein secretion into the extracellular or intracellular space (Bahn et al., 2003; Lee et al., 2003). It is noteworthy that some sPLA2s from plants have the sequences KTEL, KFEL, and KLEL at the C-terminal which are similar to the endoplasmic reticulum retention sequences KDEL and HDEL reported for animals (Matsushima et al., 2003). Even when this putative KxEL endoplasmic reticulum (ER) retention sequence (Pagny et al., 2000; Seo et al., 2008) is present in some plant sPLA2, the biochemical significance is still unknown (see Supplementary Figure S1).

### GmsPLA2s Gene Family, Classification, and Domain Structure

In G. max, five sPLA2s isoforms were identified (Mariani et al., 2012), named as GmsPLA2-XIA-I, GmsPLA2-XIA-II, GmsPLA2-XIB-I, GmsPLA2-XB-II, and GmsPLA2-XIB-III. Detailed information about the genes and proteins are shown in Table 3. As indicated above, the extension of the N-terminus of the mature protein is crucial for the activity, we show in Supplementary Figure S5 all the sequences of the sPLA2s of known plants with their signal sequence and their point of theoretical cut using the programs available online.

Moreover, the genes encoding for GmsPLA2-XIA-I and GmsPLA2-XIB-I are located in chromosome I, GmsPLA2-XIA-II y GmsPLA2-XIB-II are positioned in chromosome 7 and the gene of GmsPLA2-XIB-III is located in chromosome 8. Whereas GmsPLA2-XIB-I, GmsPLA2-XIB-II, and GmsPLA2-XIB-III possess three introns and four exons, the genes of GmsPLA2-XIA-I and GmsPLA2-XIA-II have two introns and three exons, respectively (see Supplementary Material in Mariani et al., 2012). These facts are indicative that during the course of evolution events of divergence and duplication might have occurred as it was suggested previously for AtsPLA2s (Lee et al., 2005).

All sPLA2s sequences found in plants hold a PA2c (SMART accession number SM000851) domain that contains the highly conserved Ca2+-binding loop (YGKYCGxxxxGC) (see Figure 2). The active site motif (DACCxxHDxC) that holds the highly conserved HIS/ASP pair (Laigle et al., 1973) corresponds to position 49/50 for GmsPLA2-XIA-I, 47/48 for GmsPLA2-XIA-II and 62/63 for GmsPLA2-XIBs whereas for AtsPLA2x and AtsPLA2y, it corresponds to the position 62/63 and 7/48, respectively (Mansfeld et al., 2006).

However, there is a dissimilarity that remains unclear in the HIS/ASP of the catalytic dyad in sPLA2s from plants compared with those found in animals (Mansfeld et al., 2006). It was proposed that water molecules assist in the Ca2+ coordination

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at the HIS48-ASP49 active site in bovine pancreatic \textit{bpsPLA}_2 (Bahnson, 2005), the roles of ASP99 in this \textit{sPLA}_2 (Kumar et al., 1994), and ASP64 in bee venom \textit{sPLA}_2 (West et al., 2013) were also claimed to take part in the hydroxyl-imidazole-carboxylate motif (Anand et al., 1996). However, for \textit{sPLA}_2 plant enzymes this important catalytic residue is replaced by an HIS or an ASN residue in enzymes from group XIA and by a SER or an ASN in those enzymes belonging to group XIB (Mansfeld et al., 2006) as shown in the alignment in Figure 3. Mansfeld et al. (2006) demonstrated that SER, ASN, or HIS in plant \textit{sPLA}_2s may fulfill the catalytic role assigned to ASP in animal’s \textit{sPLA}_2s (Mansfeld et al., 2006). Sequence alignment also reveals that, contrary to \textit{Os}sPLA\textit{i}s, the ASP residue of the highly conserved HIS/ASP catalytic dyad of the animal counterpart is replaced by an HIS residue in the durum wheat \textit{TdsPLA}_2 isoform 1, and by an ASN residue in all of the others durum wheat \textit{TdsPLA}_2 isoforms (Verlotta et al., 2013), see Figure 3 for more details of other \textit{sPLA}_2s from plants. Even though the comparison showed low homology among them in the overall amino acid sequences, both the catalytic site and the Ca\textsuperscript{2+} binding loop are highly conserved (Figure 3). Other relevant conserved residues within the Ca\textsuperscript{2+} binding loop are the two TYR and two GLY residues which are involved in the hydrogen bonding network reported for both animal and plant \textit{sPLA}_2s (Lee et al., 2005). A more perusal view of this domain offers additional information. The more conserved domain YGKYCG seems not to be exclusive, a change in the second TYR residue was observed for \textit{TdsPLA}_2-I changing to YGKFCG. Also, the following hydrophobic domain mainly formed by the LL pair may be VL, IL, IM, VS, IG, or VG (see Figure 3). However, the putative role of these differences on calcium affinity or phospholipase activity was not elucidated yet.

The mature proteins of both groups XIA and XIB contain 12 CYS residues (Figure 3) known to form six structural disulfide bonds that also are present in the same position as other known \textit{sPLA}_2s from plants (Mansfeld et al., 2006). CYS residues are essential for secreted \textit{sPLA}_2s and it has been shown to play a relevant role in the structural stability in mature \textit{sPLA}_2s (Six and Dennis, 2000; Mariani et al., 2015b).

The HIS residue (at position 49 in \textit{GmsPLA}_2-XIA-I, 47 in \textit{GmsPLA}_2-XIA-II and at 62 in \textit{GmsPLA}_2-XIB-II, -II, and -III) was suggested to play a crucial role in the nucleophilic attack at the sn-2 bond in the glycerol backbone of phospholipids for all \textit{sPLA}_2s (Six and Dennis, 2000; Berg et al., 2001; Burke and Dennis, 2009). All plants \textit{sPLA}_2s are low MW enzymes (12–18 kDa) with the exception of \textit{Cs}sPLA\textit{\beta} from Citrus that has an unexpected high MW (Table 4). The theoretical isoelectric points (\textit{pI}) for each \textit{sPLA}_2 are shown also in Table 4. As it can be observed, four of the putative \textit{GmsPLA}_2s are rather acidic or neutral (\textit{GmsPLA}_2-XIA-I, \textit{GmsPLA}_2-XIB-I, -II, and -III) as reported for \textit{sPLA}_2s isolated from Bothrops \textit{diporus} venom (de Haas et al., 1968; Daniele et al., 1997). Acidic \textit{sPLA}_2s were also reported for some enzymes found in the Crotalinae subfamily (dos Santos et al., 2011) and those found in rice (isoforms I and III) (Lee et al., 2005).
### Table 4: Molecular weight, isoelectric point, and specific activity of different sPLA2s from plants.

| Origin              | Name              | Mature protein MW (kDa) | pI      | Reported activity (µmol min⁻¹ mg⁻¹ protein) and substrate | References                      |
|---------------------|-------------------|-------------------------|---------|----------------------------------------------------------|----------------------------------|
| A. thaliana (arabidopsis) | AtsPLA₂-α        | 14.2                    | 7.7     | 16.7 (DOPC)                                              | Lee et al., 2005; Mansfeld and Ulbrich-Hofmann, 2007 |
|                     | AtsPLA₂-β        | 16.3                    | 8.2     | 0.63 (PC)                                                | Lee et al., 2003, 2005            |
|                     | AtsPLA₂-γ        | 17.5                    | 8.3     | NR                                                       | Bahn et al., 2003; Lee et al., 2005 |
| D. caryophyllus (carnation) | DcsPLA₂        | 12.4                    | 6.9     | NR                                                       | Lee et al., 2005                  |
| R. communis (castor bean) | RcsPLA₂          | 14                      | 6.3⁺    | 52.3 pmol min⁻¹ mg⁻¹ (l¹⁴C)18:1-PC                        | Bayon et al., 2015                |
| C. sinensis (orange) | CssPLA₂α         | 17.1                    | 6.9⁺    | 0.013² arachidonoyl Thio-PC                              | Domingues et al., 2007; Liao and Burns, 2010 |
|                     | CssPLA₂β         | 31.6                    | 8.1⁺    | 0.013² arachidonoyl Thio-PC                              | Liao and Burns, 2010              |
| U. glabra (elm)     | UgSPLA₂          | 13.9                    | NR      | 90 (PCPC)                                               | Stahl et al., 1998; Lee et al., 2005 |
| G. max (soybean)    | GmsPLA₂-XIA-I    | 12.3                    | 6.9     | 0.44 (DLPC)                                              | Mariani et al., 2012, 2015b       |
|                     | GmsPLA₂-XIA-II   | 12.6                    | 7.4     | NR                                                       | Mariani et al., 2012, 2015b       |
|                     | GmsPLA₂-XIB-I    | 13.9                    | 5.7     | NR                                                       | Mariani et al., 2012, 2015b       |
|                     | GmsPLA₂-XIB-II   | 13.9                    | 5.7     | 0.25 (PC)                                                | Mariani et al., 2012, 2015b       |
|                     | GmsPLA₂-XIB-III  | 14                      | 6.8     | NR                                                       | Mariani et al., 2012, 2015b       |
| L. usitatissimum (flax) | LusPLA₂-I      | 17.9                    | 6.7     | ~2 (PC₂₄₅₆)                                             | Gupta and Dash, 2017              |
| P. somniferum (opium) | PsaPLA₂          | 14                      | 6.9     | ~7 (DOPC)                                               | Jablonicka et al., 2016           |
| O. sativa (rice)    | OssPLA₂α         | 12.9                    | 7.9     | 145 (sn1-palmitoyl-sn2-palmitoyl-PC)                      | Stahl et al., 1999; Lee et al., 2005 |
|                     | OssPLA₂-β        | 13.8                    | 5.5     | 145 (sn1-palmitoyl-sn2-palmitoyl-PC)                      | Stahl et al., 1999; Lee et al., 2005 |
|                     | OssPLA₂-βIII     | 13.5                    | 4.8     | NR                                                       | Lee et al., 2005                  |
| N. tabacum (tobacco) | Nt1PLA₂         | 17.0                    | 8.57    | 1.2 (POPC)                                              | Fujikawa et al., 2005             |
|                     | Nt2PLA₂         | 12.7                    | 6.8⁺    | NR                                                       | Lee et al., 2005                  |
| L. esculentum (tomato) | LesPLA₂         | 13.9                    | 6.9     | NR                                                       | Lee et al., 2005                  |
| T. durum (durum wheat) | TdsPLA₂-α        | ~14⁺                    |         | 1.56² (PC₂₄₆₇)                                          | Verlotta et al., 2013            |
|                     | TdsPLA₂-β        | ~15.7⁺                  |         | 1.56² (PC₂₄₆₇)                                          | Verlotta et al., 2013            |
|                     | TdsPLA₂-βIII     | ~13.9                   | 4.5⁺    | 3.2 (PC₂₄₅₆)                                           | Verlotta and Trono, 2014          |
|                     | TdsPLA₂-βIV      | ~17⁺                    | 1.55³ (PC₂₄₅₆)                                     | Verlotta et al., 2013            |
| Z. mays (maize)     | ZmsPLA₂         | 14.3⁺                   | 5.43⁺   | NR                                                       | This review                      |

*Indicates the pI or MW calculated by using the online interface https://web.expasy.org/compute_pi/. +The mixed enzyme activity was determined from an 8-day-old rice shoots extract (Stahl et al., 1999). §Corresponds to full-length sequence (Verlotta et al., 2013). The enzyme activity of all isoforms (per gram of dry extract) was determined from a direct orange (Liao and Burns, 2010) and wheat (Verlotta et al., 2013) extract. NR, not reported.

On the other hand, the expected pI of GmsPLA₂-XIA-II is slightly alkaline similar to those of all the sPLA₂ found in Arabidopsis (Lee et al., 2005) and Papaver somniferum (Jablonicka et al., 2016); whereas other sPLA₂s have a pI almost neutral as those found for carnation and tomato (Lee et al., 2005).

The functional role of the diverse pIs found in different sPLA₂s has not clearly been elucidated yet.

Another relevant domain information is that the enzymes from the different subgroups differ in the third Ca²⁺ coordinating amino acid, being a GLY residue in subgroup XIA or LEU residue in subgroup XIB (see Figure 3). The ASP located upstream in the sequence of the common HIS/ASP catalytic dyad found in animal sPLA₂ does not correlate in the counterpart found in plants. Instead of this additional ASP residue, the plant enzymes that belong to group XIA contain an HIS residue, and the enzymes belonging to group XIB contain either a SER or an ASP residue (Mansfeld et al., 2006). The functional role of these differences...
with regard to the catalytic properties has not been completely elucidated yet.

**GmsPLA2**s CLASSIFICATION IN THE sPLA2 SUPERFAMILY

Secretory phospholipases in plants superfamily is composed of multiple members represented by multiple isoforms distinguishable by their structural, catalytic and physiological characteristics. sPLA2 are within the most populated group of PLA2 in nature which in turn is classified into 15 subgroups (Six and Dennis, 2000). In this context, the plant sPLA2s were classified into a separate group (group XI) (Meneghetti and Maggio, 2013), which, in turn, could be subdivided into two categories named XIA and XIB because of differences in MW and deviating sequences in the N- and C-terminal regions of the mature enzyme (Six and Dennis, 2000).

Figure 4 shows the phylogenetic classification into the two subgroups of all the sPLA2s from plants known until now. This way, GmsPLA2-XIA-I and -II are taking part of group XIA, which includes AtsPLA2-γ, AtsPLA2-β, AtsPLA2-δ, O. sativa isoform I, N. tabacum isoform I, T. durum isoform I, C. sinensis isoform β, and L. usitatissimum isoform II. Whereas two of the enzymes of G. max correspond to the subgroup XIA, three are grouped in the subgroup XIB (Mariani et al., 2012) named as GmsPLA2-XIB-I, -II, and -III together with AtsPLA2-α, O. sativa-II, -III, and -IV, D. caryophyllus, N. tabacum isoform II, Z. maize, R. communis isoform α, P. somniferum, T. durum-II, -III, and -IV, L. esculentum, C. sinensis isoform α, and L. usitatissimum isoform I.

The data show a close evolutionary relationship among all sPLA2s from plants (see Figure 4). The highest level of similarity in amino acid sequences was observed between GmsPLA2-XIA-I and GmsPLA2-XIA-II, being of 95.5%, whereas between GmsPLA2-XIB-I and GmsPLA2-XIB-II the level of similarity is of 94.5% (Mariani et al., 2012) (see Supplementary Figure S2). Moreover, between LePLA2 and NtsPLA2-II the level of similarity is of 90.4% and between TdsPLA2-I and OssPLA2-I, GmsPLA2-II and GmsPLA2-III, PsPLA2 and RosPLA2-α and AtsPLA2-δ and AtsPLA2-γ the levels of similarity are of 89.9, 87.3, 83.7, and 82.7%, respectively.

TRIDIMENSIONAL STRUCTURE

Although the sPLA2 sequences from different sources differ significantly, the tridimensional structures have many features...
in common. There are more than 40 sPLA₁S entries in the Protein Data Bank (PDB)² from all sources. Native and complex structures of sPLA₁S simulated with mimic substrate have helped to identify the catalytically important residues involved in the active site (Pan et al., 2002).

The tridimensional structure of many sPLA₁S, such as porcine pancreas or bee venom (Dijkstra et al., 1984; Scott et al., 1990), has been elucidated by X-ray crystallography which revealed a common, rigid and highly conserved region with a similar tridimensional architecture compared with those from plants. The active site is not directly accessible to the aqueous phase and is within a rather local hydrophobic environment denoted as “i-face” that allows the interaction with the substrate in its monomeric form (Dijkstra et al., 1981). The putative residues involved in the “i-face” of some sPLA₁ from plants are shown in Table 5.

One of the first sPLA₁ “i-face” identified was for the secreted pig pancreatic enzyme (Bai et al., 2008). In Gm sPLA₁-XIA-I the residues found in the putative “i-face” are VAL18, GLY19, VAL28, HIS49, HIS64, LEU101, ALA102, ILE103, LEU104, LEU105, and LEU108. Table 5 shows the putative amino acids proposed to be in contact with the membrane for different sPLA₁ enzymes.

The binding of sPLA₁ to the membrane is energetically favorable (Table 6) and, keeping in mind that most of the residues situated in the i-face are hydrophobic, the overall domain constitutes a hydrophobic environment that surrounds the active catalytic site. Hydrophobic side chains of the residues forming the “i-face” would be able to partition to the hydrophobic core, which allows the anchoring of the enzyme to the membrane, excluding water molecules in the region surrounding the active site and the diffusion of the substrate to the pocket of the active site to be hydrolyzed. The general molecular conformation proposed for plants sPLA₁ is in agreement with the general vision proposed for secreted phospholipases of animal source (Scott et al., 1990).

Physically, the soluble sPLA₁ protein must penetrate the phospholipid interface to exert its action. Therefore, the successful binding surface is located where the substrate is a prerequisite in the catalytic cycle, and this property can determine some specific characteristics of the enzyme activity. However, there are a limited number of charged residues in the flat topography of the “i-face” (see Table 5) that could modulate further interaction with the interface of the substrate in a way which has not been fully elucidated yet (Jain and Berg, 2006).

It is important to note that even when the energetic to membrane binding is favorable according to the available on-line calculation program (Lomize et al., 2012) used for some sPLA₁S, the residues involved in the “i-face” differ for the same enzyme if a different approach is used instead (compare Tables 5, 6).

To date, only few structures corresponding to sPLA₁S from plants were reported in the PDB or in the Protein Model Database (PMDB)³ and correspond to O. sativa (rice) isoform II (PDB 2WG7), which belongs to the group XIB, and its tertiary structure was recently determined by X-ray crystallography to 2.0 Å resolution (Guy et al., 2009). Moreover, homology modeling and molecular dynamics were used to elucidate the structure of sPLA₁, isoform α from Arabidopsis (Mansfeld et al., 2006) but its PDB is not available. The predicted models of LusPLA₁ proteins were elucidated and submitted to PMDB identified as PM0080416 (LusPLA₁-I) and PM0080415 (LusPLA₁-II) (Gupta and Dash, 2017). The structure of Gm sPLA₁-XIA-I was modeled by using homology modeling and molecular dynamics (Mariani et al., 2012) and also Gm sPLA₁-XIB-II by using a similar methodology (see Figure 5, modeled structures in PDB format were not uploaded in the PDB). The data corresponding to Pig pancreatic (Sus scrofa), Naja naja (Indian cobra), Naja sagittifera (Andaman cobra venom) are also indicated in Table 6 for comparison in order to include sPLA₁ able to hydrolyze aggregate lipids structured in a high packing organization, as it occurs with sPLA₁ from cobra venom, or only at low packing as it certainly happens with sPLA₁ from pig pancreas (see below and Table 10).

The structure of rice sPLA₁ shows that the half N-terminal chain contains mainly structured loops, including the conserved calcium binding loop domain together with two short anti-parallel β-strands. The half C-terminal is folded into three anti-parallel α-helix, in which two of them are highly conserved among others sPLA₁S, containing the crucial catalytic HIS residue and the calcium binding/coordinating ASP residues (Guy et al., 2009). This overall general folded conformation seems to be shared by almost all known sPLA₁ from plants. The complete putative mature structure of Gm sPLA₁-XIA-I protein was reported using homology modeling and molecular dynamics simulations (Mariani et al., 2012). The most mobile regions are the N- and C-terminal, followed by the loops in residues 74–85, 53–62, 34–37 that connect, respectively, the last two helices, the first with the second helix, and the last beta-sheet with the first helix (see Figure 5). As other sPLA₁S in the family, the dominant secondary structure is the α-helix, with only a small portion of beta sheet with abundant regions containing turns and bends. The observations indicate that the terminal helix is rather a dynamic region and has three principal conformations: one fully helical, other with the last seven residues in coil, and the third one with a kink plus coil (Mariani et al., 2012). As noted before, this behavior can be attributed to a low number of hydrophobic

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2https://www.rcsb.org/

3http://srv00.recas.ba.infn.it/PMDB/main.php

| sPLA₁ name       | Proposed amino acids in the “i-face” | References                                                                 |
|------------------|--------------------------------------|---------------------------------------------------------------------------|
| PpsPLA₁ (Group IB) | L², V², R³, L¹³, M²⁰, L³¹, and Y⁶⁹   | Kuipers et al., 1991                                                       |
| BpsPLA₁           | L², V², F, β, P, P₂², L₂₁, L₆₃, and Y⁶⁹ | Yu et al., 1999b                                                           |
| GmsPLA₁-XIA-I     | V₁₈, G₁₀, V₂₈, H₄₉, H₁₀₁, A₁₀₂, L₁₀₃, L₁₀₄, L₁₀₅, and L₁₀₈ | Mariani et al., 2012                                                       |
| GmsPLA₁-XIB-II    | F₂₅, S₂₇, L₂₁, V₁₁₂, A₁₁₆, L₁₁₉, V₁₂₃, L₁₂₄, and P₁₂₇ | Obtained by using the on-line platform OPM (Lomize et al., 2012) |
| Os sPLA₁-II       | A²⁹, P₃₀, V₆⁵, V₇₂, and L₄¹           | Guy et al., 2009                                                           |

Pp, P. pancreas; Bp, B. pancreas; Gm, G. max; Os, O. sativa.
TABLE 6 | Orientation of different sPLA2s at the membrane interface.

| Protein                  | Depth/hydrophobic thickness (Å) | ΔGtransf (kcal/mol) | Tilt angle | Embedded residues |
|--------------------------|---------------------------------|--------------------|------------|------------------|
| GmsPLA2-XIB-II           | 2.8 ± 0.9                       | −10.4              | 71 ± 4°    | F20, S27, L31, V112, A116, L119, V123, L124, and P127 |
| GmsPLA2-XIA-I            | 1.0 ± 2.8                       | −1.0               | 69 ± 20°   | P114             |
| OssPLA2-I                | 4.1 ± 0.5                       | −10.6              | 71 ± 2°    | G3, L5, A25, L28, V30, G31, 116, R120, and D121 |
| LusPLA2-I                | 4.1 ± 0.6                       | −7.1               | 86 ± 3°    | A27, A29, V30, P32, and L33 |
| LusPLA2-II               | 1.9 ± 1.2                       | −5.5               | 86 ± 26°   | F24 and L102     |
| Naja sagittifera NsPLA2  | 1.6 ± 0.4                       | −4.3               | 85 ± 2°    | D32 and K65      |
| Naja naja NsPLA2         | 3.6 ± 0.3                       | −6.7               | 86 ± 2°    | Y13, W19, W61, and F64 |
| Pig pancreatic PpsPLA2   | 2.4 ± 2.5                       | −1.9               | 32 ± 16°   | L64              |

Structure were modeled in a similar way than that described for GmsPLA2-XIA-I in Mariani et al. (2012), the PMDB accession number is PM0082160. Structure modeled in Mariani et al. (2012), the PMDB accession number is PM0082161. Obtained from PDB 2WG7. PMDB identified as PM0080416 (LusPLA2-I) and PM0080415 (LusPLA2-II). Crystal structure of Naja sagittifera was reported in Jabeen et al. (2005). PDB 1MH8. Naja naja NsPLA2. PDB 1A3D. Pig pancreatic PpsPLA2. PDB 1PIR.

contacts of this region, a high aqueous exposed area and the presence of a highly flexible GLY98 residue (Mariani et al., 2012).

The active site of the sPLA2 protein contains a crucial calcium ion cofactor commonly present in other plant sPLA2s (Mansfeld et al., 2006; Guy et al., 2009) that is important in the catalytic mechanism and is a requisite for full enzyme activity. The HIS-ASP pair constitutes the active center and the calcium binding loop (see Figure 3) is essential for the proper function of the enzyme (Scott et al., 1990). All sPLA2s catalyze the hydrolysis through the same mechanism: an abstraction of a proton from a water molecule followed by a nucleophilic attack on the sn-2 bond position of the diacylglycerolphospholipid (Jorgensen et al., 1983; Berg et al., 2001). NMR structural studies of porcine pancreas sPLA2 show that the N-terminus is flexible with no defined structure in solution, unlike what it was evidenced by crystallography. It was hypothesized that this flexibility in solution would be related to the near null activity against monomeric substrate form [more unstructured unbound state (van den Berg et al., 1995)].

ENZYMATIC PROPERTIES OF PLANTS sPLA2s

Optimum Conditions for Plants sPLA2s Catalysis

The sPLA2s from N. tabacum and elm have optimum pH in the range of 8–10 and 8–9, respectively (Stahl et al., 1999; Fujikawa et al., 2005). In Arabidopsis thaliana sPLA2s catalyzing the hydrolysis of diacylglycerolphospholipids (DLP) showed optimum pH ranges for the activities are pH 6–11, 6–7, 7–9, and 8–9 for AtsPLA2-α, -β, -γ, and -δ, respectively (Lee et al., 2005). Nevertheless, a similar situation was found for almost all the sPLA2s found in plants or animals. The pH optimum was at around 7 for GmsPLA2-XIA-I and -XIB-II (see Table 7), when using mixed micelles of DLPC:Triton X-100 as substrate in presence of calcium 10 mM. The optimum pH for pancreatic sPLA2 was reported to be 8 (de Haas et al., 1968; Fujikawa et al., 2005) similar to that reported for bee venom (Daniele et al., 1997). For human non-pancreatic PL2 optimum pH is in between 8 and 10 (Kramer et al., 1989). However, it should be mentioned that different substrates (including different aggregation presentation of substrate) have been used to determine optimum pH for the different sPLA2s reported in the literature.

Only few sPLA2s were investigated about the optimum temperature and stability. GmsPLA2s-XIA-I and -XIB-II demonstrated to be very stable when increasing the temperature (Mariani et al., 2015b) as previously determined by using an sPLA2 homogenate (Minchiotti et al., 2008). This proved that these enzymes are highly resistant to temperature denaturation due in part to the disulfide bridges that are postulated to be involved in the stability of sPLA2s (Berg et al., 2009; Murakami et al., 2010). Table 7 shows the optimal temperature reported for several sPLA2s from plants.

The optimum calcium concentrations for activity of GmsPLA2-XIA-I and -XIB-II are in the micromolar range using DLPC:Triton X-100 mixed micelles as substrates (Table 7). This micromolar calcium requirement is rather unusual for sPLA2s enzymes that mostly possess millimolar requirement.
TABLE 7 | Optimum requirements deduced for catalytic activity of the different sPLA2-s found in plants.

| Source          | Name               | pH  | Calcium requirement* | T (°C) | References                        |
|-----------------|--------------------|-----|----------------------|--------|-----------------------------------|
| A. thaliana     | AtsPLA2-α          | 6-11| mM                   | 30-40  | Lee et al., 2005; Mansfeld et al., 2006; Mansfeld and Ulbrich-Hofmann, 2007 |
| Arabidopsis     | AtsPLA2-β          | 6-7 | > 0.5 mM             | 30     | Lee et al., 2003, 2005            |
|                 | AtsPLA2-γ          | 7-9 | > 0.5 mM             | 30     | Bahn et al., 2003; Lee et al., 2005 |
| R. communis     | RcsPLA2-α          | 8   | 10 mM                | 30     | Bayon et al., 2015               |
| S. bicolor      | CcsPLA2-α          | 7.4 | 10 mM                | 25     | Liao and Burns, 2010             |
| Orange          | CcsPLA2-β          | 7.4 | 10 mM                | NR     | Liao and Burns, 2010             |
| D. caryophillus | DcsPLA2            | NR  | NR                   | NR     | –                                 |
| U. glabra       | ElmPLA2            | 8-9 | 10-15 mM             | 30     | Stahl et al., 1998               |
| G. max (soybean)| GmPLA2-XIA-I       | 6-7 | > 1 mM               | 40-60  | Mariani et al., 2015b            |
|                 | GmPLA2-XIA-II      | NR  | NR                   | NR     | –                                 |
|                 | GmPLA2-XIB-I       | NR  | NR                   | NR     | –                                 |
|                 | GmPLA2-XIB-II      | 6-7 | > 1 mM               | 40-60  | Mariani et al., 2015b            |
| L. usitatissimum| LusPLA2-I          | 9   | 1 mM                 | NR     | Gupta and Dash, 2017             |
|                 | LusPLA2-II         | 9   | 1 mM                 | NR     | Gupta and Dash, 2017             |
| P. somniferum   | PrsPLA2            | 7   | NR                   | 37     | Jabionicka et al., 2016           |
| O. sativa (rice)| OssPLA2-α          | 8   | 10 mM                | 30     | Stahl et al., 1999; Guy et al., 2009 |
|                 | OssPLA2-β          | 8   | 10 mM                | 30     | Stahl et al., 1999; Guy et al., 2009 |
|                 | OssPLA2-δ          | NR  | NR                   | NR     | –                                 |
| N. tabacum      | N1sPLA2            | 8-10| < 1 mM               | NR     | Fujikawa et al., 2005, 2011       |
| Tobacco         | N2sPLA2            | –   | –                    | –      | NR                                |
| L. esculentum   | LesPLA2            | NR  | NR                   | NR     | NR                                |
| T. durum        | TdPLA2-I           | 9   | > 2 mM               | 25     | Verlotta et al., 2013            |
| Durum wheat     | TdPLA2-II          | 9   | > 2 mM               | 25     | Verlotta et al., 2013            |
|                 | TdPLA2-III         | 9   | 1 mM                 | 25     | Verlotta et al., 2013; Verlotta and Trono, 2014 |
| Z. mays (maize) | ZmPLA2             | –   | –                    | NR     | –                                 |

*For Arabidopsis sPLA2s-β, -γ, and -δ, a µM requirement was reported without specifying the precise concentration. NR, not reported.

(Six and Dennis, 2000). Moreover, the same behavior was observed for the activities of AtsPLA2-β, -γ, and -δ (Lee et al., 2005). It is important to remark that none of these secreted enzymes (either from animals or plants) exhibit activity in absence of calcium. Particularly, for AtsPLA2-α the activity augmented as the calcium concentration increased up to 10 mM and for elm sPLA2 the range of calcium concentration for optimal activity was around 10–15 mM CaCl₂ (Stahl et al., 1998; Lee et al., 2005; Mansfeld and Ulbrich-Hofmann, 2007). However, to achieve 50% of maximal enzyme activity a concentration of 0.5 mM CaCl₂ was sufficient, at least, for these two latter enzymes. The maximal activity for sPLA2 from N. tabacum was detected above 1 mM CaCl₂. This behavior is similar to that observed for the most animal sPLA2s, which require millimolar concentrations of Ca²⁺ and have no activity in the absence of this cation (Six and Dennis, 2000; Fujikawa et al., 2005). Even when it is evident the molecular differences among the enzymes in the sPLA2 family, the absolute requirement of Ca²⁺ for hydrolysis is indicative that all of them share a common mechanism for lipid hydrolysis. For durum wheat sPLA2 the activity continuously increased as Ca²⁺ concentration increased with a plateau close to 2–4 mM CaCl₂, even though a 300 µM CaCl₂ was sufficient to reach 50% of the maximal activity (Verlotta et al., 2013) (see Table 7).

The differences in the activity reported from many authors for plant sPLA2s is not easy to compare in absolute terms. Usually the reported activity values are informed as specific activities (µmol of hydrolyzed lipid.min⁻¹.mg of protein⁻¹) and this quantity may be affected by many factors. Among the main factors that can affect the sPLA2 activity can be mentioned (i) inherent deficiencies in the folding of recombinant enzymes, (ii) additional tags at the N-terminus, and (iii) the lack of standardization of substrate offered to the enzyme (lipid monolayers, micelles,
SUVs, presence of detergents mixed with the lipid substrate, etc.). However, taking into account these precautions the activity of the reported enzymes could be compared although different substrates and systems were used in the assays (see Table 4).

Conformational Stability of sPLA2s

It is known that CYS residues are essential for the structural stability of sPLA2 and it has been shown to play an important role in the structural stability of the mature enzyme (Six and Dennis, 2000; Welker et al., 2011). In animals, sPLA2s contain between 10 and 16 CYS that have the potential to form 5–8 intramolecular disulfide bridges (Schaloske and Dennis, 2006). In contrast, all sPLA2s reported from plants have 12 CYS that can form 6 disulfide bridges (see Table 1). It is known that some sPLA2 from animals (especially type I and II), are rather stable upon heating compared with cytoplasmic cPLA2 (Mazereeuw-Hautier et al., 2000). Resistance to heating for sPLA2 from plants was reported for some enzymes indicating a similar behavior to that observed for animal sPLA2. The structural stability for durum wheat sPLA2 was demonstrated by the resistance to high temperatures (87% of the activity was retained after treatment of the crude leaf extract at 100°C for 15 min), see (Verlotta et al., 2013). Recombinant AtsPLA2α and AtsPLA2β retained 80–95% of their activities following 5 min treatment in boiling water (Lee et al., 2005), and a similar result was obtained for sPLA2 purified from elm seeds (Stahl et al., 1998). Moreover, for GmsPLA2-XIIA-I and GmsPLA2-XIB-II preserved the activity after heating 5 min at 80°C (Mariani et al., 2015b).

The main reason for the scarceness of information on recombinant plant sPLA2s may be attributed to the low expression yields obtained with the different protocols currently used and the strong propensity of the recombinant enzymes to aggregate (Mansfeld et al., 2006). The generally lower yields of the purified enzymes from inclusion bodies might be an indication for a higher fraction of misfolded and/or aggregated protein after the renaturation process. This may be the reason of different Vmax or specific activity values obtained when studying kinetic parameters in sPLA2 recombinant enzymes from plants (see Table 9).

In bee venom sPLA2 (BvPLA2), it was reported that the formation of disulfide bonds is not essential for correct re-folding of the protein and an active enzyme form can be reobtained even from the completely denatured and reduced state (Welker et al., 2011). It is known that, in contrast to the seven disulfide bonds present in porcine pancreas enzyme (PpPLA2), all five disulfide bonds of BvPLA2 are essential for conformational stability and contribute to the activity (Welker et al., 2011). In the case of bacterial sPLA2 from Streptomyces violaceoruber, it possesses only two disulfide bridges (Sugiyama et al., 2002) which were sufficient to be active comparable to animal or plant sPLA2s (Yunes Quarinho et al., 2015).

In sPLA2 from A. thaliana, the removal of disulfide bonds increased the proteolytic susceptibility of the native proteins whereas the stability decreased (Mansfeld et al., 2014). Regarding GmsPLA2s, it was demonstrated that the calcium ion also contributes to keep the protein folded in its native structure.

This effect was observed by two independent assays using dynamic simulations and intrinsic fluorescence experiments (Mariani et al., 2012, 2015b).

The comparison of the data obtained on bovine pancreatic sPLA2, bee venom sPLA2, and porcine pancreatic sPLA2 with those obtained on sPLA2s from plants suggests that conserved disulfide bonds in those homologous proteins are important to keep the conformational architecture and stability. However, with the recompiled information, it is almost clear that not all the disulfide bridges are needed for the protein to be active, but are necessary for a protein correct folding.

INTERFACIAL CATALYSIS ACTIVATION

Phospholipids are constituents of biological membranes, so a very important prerequisite step to perform the lipolytic action of sPLA2 is the interaction with the amphipathic nature of these interfaces; and in turn, determine the catalytic properties of the organized substrate (Jain and Berg, 2006). The interfacial binding step is crucial for enzymatic action of sPLA2, and it is mediated by a region of the protein often referred to as i-face (see above), also reported as IRS, the interfacial recognition site (Tatulian, 2001). The i-face or IRS is not a proper “site” or a flat face, it is rather a 3D domain with the confluence of several residues that crowns and precedes the catalytic site, giving an adequate environment for the catalysis, and also help keeping the enzyme attached to the membrane where the hydrolytic reaction takes place. The proper intimate contact of the i-face of sPLA2s with the interface is essential to provide the substrate access to the active site. Interfacial activation is a concept that means an adequate contact between the catalytic active site and the i-face modulating the catalytic activity (Scott et al., 1990; Tatulian et al., 2005; Jain and Berg, 2006; Winget et al., 2006).

The binding and kinetic characteristics of interfacial catalysis by sPLA2 depend upon the organization and dynamics of the interface. The overall rate of catalytic turnover is not only determined by the kinetics at the interface, but also by the binding/desorption equilibrium kinetics of the enzyme with the interface (Ramirez and Jain, 1991). Hence, the hydrolysis of the organized substrate can occur in two extreme distinct modes: (i) in the scooting mode of catalysis, that requires that the enzyme remains bound at the interface between several catalytic turnover cycles and, (ii) in the pure hopping mode, where the binding and the desorption of the bond enzyme occur during each catalytic turnover cycle leading to a jumping mechanism (Jain et al., 2009) (see Supplementary Figure S4 for more details and a schematic description of both mechanism of lipids hydrolysis induced by sPLA2).

A few mode of interfacial catalysis for sPLA2s has been reported. Moreover, in plants, we were the only in studying the catalytic mode till today. The enzyme studied in order to determine the mode of catalysis was the sPLA2 from G. max (GmsPLA2-XIIA-I) (Mariani et al., 2015b). Whereas pancreatic sPLA2 presents a scooting mode of catalysis when using anionic lipids (Berg et al., 1991), it presents a hopping mode of catalysis if the specific experimental conditions are changed to
zwitterionic lipids (Scott et al., 1994). In our hands, GmSPAL2-XIA-I acts in the hopping mode against zwitterionic lipids (Mariani et al., 2015b).

**Hydrolysis Using Micelles as Substrate Membrane Model System**

There have been some reports in the literature regarding sPLA2 activity against different substrates and in different conditions. For some sPLA2, it has been demonstrated that the hydrolysis rate is sensitive to the surface charge density of the lipid aggregates (Volwerk et al., 1986). Several kinetics studies on pancreatic as well as snake venoms and plants phospholipases have been reported in which lipid phase transition, lipid membrane curvature, and composition may modulate the lipolysis (Wilschut et al., 1978; Bell and Biltonen, 1989; Bell et al., 1996; Leidy et al., 2004). However, it should not be forgotten, that sPLA2 has optimum of lipid packing for hydrolysis, i.e., that some enzymes have the ability to hydrolyze lipid in a low packing organization (low lateral pressure in lipid monolayers more compatible with micelles) but others also have optimum condition of hydrolysis at high lateral pressure in monolayers compatible with liposomes or biological membranes (Ramirez and Jain, 1991; Yunes Quartino et al., 2015).

Usually, short-chain zwitterionic phospholipids have been employed as substrates in single component systems (de Haas et al., 1971; Wells, 1972) or, for the case of long-chain phospholipids, they were mixed with neutral detergents (Dennis, 1973b; Yu et al., 1999a; Mansfeld and Ulbrich-Hofmann, 2007). Moreover, the activity is generally increased when the lipid substrate forms mixed micelles in presence of detergents (Dennis, 1973a; Dennis et al., 1981). The effect of enzyme immobilization on the sPLA2 kinetics was also reported (Madoery et al., 1999). Description and kinetics properties of sPLA2 from plants have been more frequent in their recombinant counterpart after appropriate expression, purification, and folding protocols (Bahn et al., 2003; Ryu et al., 2003; Fujikawa et al., 2005; Mansfeld and Ulbrich-Hofmann, 2007; Mariani et al., 2012) compared with their equivalent found in animals sPLA2s. The reason of this is due to the relative high amounts of the latter proteins found in their respective natural sources (venoms and pancreatic juice) and, therefore, it allows an efficient purification of the mature forms of sPLA2. However, few studies using purified plant enzymes were reported from elm seeds (Stahl et al., 1998) and of G. max (Minchiotti et al., 2008).

Mammalian and plant enzymes differed in head group specificity. While some mammalian sPLA2s show high activity on anionic phospholipids (Ghomaschi et al., 1991; Bezzine et al., 2002), sPLA2s from plants preferred zwitterionic phospholipids (Mansfeld and Ulbrich-Hofmann, 2007; Mansfeld, 2009; Mariani et al., 2015b). In Table 8 we summarize the substrate lipid preference (head group or acyl chain) differences observed in some sPLA2s from plants reported in the literature (see also Table 9 additional kinetic data).

Table 9 shows the $K_m$ and $V_{max}$ values determined and reported for sPLA2s from different sources. As shown, we can infer that the values of $V_{max}$ could be sensitive to both the lipid substrate used in the assays and the interfacial quality of the surface in which the substrate is inserted.

**Phospholipid Hydrolysis Using Langmuir Monolayers as Membrane Model System**

The influence of substrate lipid packing on sPLA2 activities was studied for numerous authors using Langmuir-lipid monolayers performed at different surface pressures using almost exclusively sPLA2 from animal sources (Yunes Quartino et al., 2015). Moreover, to study the catalytic activity at the air-water interface the lipid monolayer technique in the “zero order” regime was used since the surface pressure is kept constant during the reaction (Panaiotov and Verger, 2000; Yunes Quartino et al., 2012) (see Supplementary Figure S3 for a schematic representation of this experimental system).

The optimum surface pressure of these enzymes to hydrolyze the lipids of the membranes differed with the origin of the sPLA2 (Ramirez and Jain, 1991; Mariani et al., 2015b; Yunes Quartino et al., 2015). GmSPAL2s were the first sPLA2s from plants to be studied with respect to their interfacial characteristics. Table 10 shows the optimum pressure determined for different sPLA2s. The optimum for plants GmSPAL2s seems to fall intermediate in between the values of “pancreatic like” enzymes that have high activity against micelles structured lipids rather bilayers (lipolytic ratio lower than 0.1) compared with toxic venom sPLA2s (lipolytic ratio higher than 1) that can hydrolyze intact cell membranes such as erythrocytes (Demel et al., 1975). Then, it may be concluded that sPLA2s from plants would have a more ubiquitous functionality, since they can be active in vitro against a rather wide range of curvature radio of structured lipid substrates (less sensitivity to the supramolecular organization).

**Auxin Effect Over sPLA2 Activity**

Studies of plant sPLA2s demonstrated that auxins play important roles in signal transduction regulating cellular processes and probably they are implicated in phospholipid signaling (Wang, 2001; Ryu et al., 2005; Scherer et al., 2010). At the cellular level, auxins control cell division, growth, extension, and differentiation (Davies, 1995). At the whole plant level, auxins play an essential role in processes such as apical dominance, lateral/adventitious root formation, tropisms, fruit set and development, vascular differentiation, and embryogenesis (Friml, 2003). A rapid increase in sPLA2 activity was first verified by treating isolated microsomes and cell cultures with auxins (Scherer and Andre, 1989; Scherer, 1990; Andre and Scherer, 1991; Scherer, 1992; Scherer and Andre, 1993; Scherer, 1996) and microsomes isolated from hypocotyls segments (Blanchet et al., 2008b). However, as the molecular mechanism of the putative effect of auxins over sPLA2s is unknown we have investigated whether these phytohormone have any direct effect over the enzyme by using simple in vitro assays.

Secretory phospholipases, like other lipolytic enzymes, are interfacial active proteins, since they access from water to the interface of the insoluble organized substrate to carry...
### TABLE 8 | Substrate preference of different sPLA₂s from plants.

| Origin                  | Name             | sn-specificity | Fatty acid preference | Head group selectivity | References                                                                 |
|-------------------------|------------------|----------------|------------------------|------------------------|-----------------------------------------------------------------------------|
| Arabidopsis             | AtsPLA₂-α, AtsPLA₂-β | sn-2 sn-2     | Linoleic > Palmitic > Linoleic | PC > PE (baja) > PG > PI (low) | Lee et al., 2005; Mansfeld and Ulbrich-Hofmann, 2007; Lee et al., 2005 |
|                         | AtsPLA₂-γ        | sn-2           | Linoleic > palmitic-oleic | PE (high)              | Bahn et al., 2003; Lee et al., 2005                                       |
|                         | AtsPLA₂-δ        | sn-2           | Palmitic-oleic > Linoleic | PE (high)              | Bahn et al., 2003; Lee et al., 2005                                       |
| Carnation               | DcSLA₂           | NR             | NR                     | NR                     | NR                                                                          |
| Castor bean             | RcsPLA2α         | sn-2           | Palmitic > ricinoleic   | PC                     | Bayon et al., 2015                                                          |
| Orange                  | CsaPLA2α         | Deducted sn-2  | NR                     | NR                     | Liao and Burns, 2010                                                        |
|                         | CsaPLA2β         | Deducted sn-2  | NR                     | NR                     |                                                                              |
| Elm                     | UgsSLA₂          | sn-2           | Oleic (C8–C12)         | NR                     | Stahl et al., 1998; Lee et al., 2005                                       |
| Soybean                 | GmSLA₂-XIA-I     | sn-2           | Lauril                  | PC                     | Mariani et al., 2015                                                       |
|                         | GmSLA₂-XIA-II    | Deducted sn-2  | NR                     | NR                     | Mariani et al., 2015                                                       |
|                         | GmSLA₂-XIB-I     | Deducted sn-2  | NR                     | NR                     | Mariani et al., 2015                                                       |
|                         | GmSLA₂-XIB-II    | sn-2           | Lauril                  | PC                     | Mariani et al., 2015                                                       |
|                         | GmSLA₂-XIB-III   | Deducted sn-2  | NR                     | NR                     | Mariani et al., 2015                                                       |
| Flax                    | LusSLA₂-I        | Deducted sn-2  | NR                     | NR                     | Gupta and Dash, 2017                                                        |
|                         | LusSLA₂-II       | Deducted sn-2  | NR                     | NR                     | Gupta and Dash, 2017                                                        |
| Linum                   | PsSLA₂           | sn-2           | Linolenic              | PC > PE                | Jablonsk&W., 2016                                                          |
| Rice                    | OssPLA₂-I        | sn-2           | NR                     | PC                     | Stahl & al., 1999                                                          |
|                         | OssPLA₂-II       | sn-2           | NR                     | PC                     | Lee et al., 2005                                                            |
| Tobacco                 | Nt1sPLA₂         | sn-1/sn-2      | NR                     | PC                     | Fujikawa et al., 2011                                                       |
|                         | Nt2sPLA₂         | sn-2           | NR                     | PC                     | Fujikawa et al., 2011                                                       |
| Tomato                  | LeiPLA₂          | sn-2           | NR                     | PC                     | Narvaez-Vasquez et al., 1999                                               |
| Durum                   | TdsPLA₂-I        | sn-2           | Non-specified          | PC                     | Verlotta et al., 2013                                                       |
|                         | TdsPLA₂-II       | sn-2           | Non-specified          | PC                     |                                                                             |
|                         | TdsPLA₂-III      | sn-2           | Palmitic               | PC                     | Verlotta and Trono, 2014                                                   |
|                         | TdsPLA₂-IV       | sn-2           | Non-specified          | PC                     | Verlotta et al., 2013                                                       |

NR, not reported.
out the lipid hydrolysis. For this reason, the activity of the enzyme is directly modulated at the interface by the supramolecular organization of the substrate summarized in the concept of "interfacial quality" [e.g., the physical state of the lipids, proper lateral packing, modulation by non-substrate lipids, "membrane lateral defects," among others (Verger et al., 2013)]. Lipid substrate-enzyme interaction involved. So, the effect of auxins can be attributable to changes in the interfacial quality of the organized substrate rather than a direct effect over the enzyme (Mariani et al., 2015a). The molecular details by which the particular mixed interfaces formed by auxins/phospholipids may modulate the sPLA₂ activity, regardless of the enzyme origin, remain to be elucidated. However, to ascertain the interfacial hypothesis of auxins over the action of sPLA₂, we further analyzed the surface properties of two auxins: IAA and IPA, i.e., the capability of these phytohormones to partition into lipid interfaces (Mariani et al., 2015a). Both IAA and IPA did not show any affinity toward lipid-clean interfaces (self-adsorption to water surface) but, very importantly, both auxins showed the ability to penetrate lipid interfaces forming stable and insoluble monolayers with phospholipids. This capability to form mixed lipid-auxin interfaces allowed the activation of two recombinants GmsPLA₂s and pancreatic sPLA₂ (Mariani et al., 2015a). The interfacial activation exerted by auxins was, regardless of sPLA₂ source, supporting the theory that at the action is at lipid-auxin interface and not a direct effect over the enzyme (Mariani et al., 2015a).

### OUTLOOK AND PERSPECTIVES

The application of biotechnology, particularly enzymes in industrial processes, is continuously growing due to its minimal environmental impact, since they produce non-toxic waste.

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**Table 9: Kinetic parameters for some reported plant sPLA₂.**

| Origin             | Kₘ₉ (mM) | Vₘ₉ (µmol.min⁻¹ mg⁻¹) | Lipid substrate used | References             |
|--------------------|----------|------------------------|----------------------|------------------------|
| GmsPLA₂-XIA-I      | 0.23     | 10.2                   | DLPC                 | Mariani et al., 2015b  |
| GmsPLA₂-XIB-II     | 1.7      | 13.9                   | DLPC                 | Mariani et al., 2015b  |
| GmsPLA₂-XIB-II     | 0.07     | 19.7                   | DLPC                 | Mariani et al., 2015b  |
| AhsPLA₂-α          | 5.7      | 29.8                   | DOPC                 | Mansfeld and Ulbrich-Hofmann, 2007 |
| TdsPLA₂            | 0.43     | 1.43 U.g⁻¹a            | PC                   | Verlotta et al., 2013  |
| Reported for animal sPLA₂ | 0.18-3.2 | NR                     | DOPC                 | Mansfeld and Ulbrich-Hofmann, 2007 |
| PpsPLA₂b           | 3.7      | 2                      | diC8-PC              | Kuipers et al., 1991   |

Kₘ₉ is expressed as specific activity. Vₘ₉ is expressed as µmol.min⁻¹ mg⁻¹. aExpressed in Units per gram of dry leaves extract. bPig pancreatic sPLA₂.

**Table 10: Parameters determined for different sPLA₂s using monomolecular films of DLPC.**

| Phospholipase A₂ origin | Optimum surface pressure | Substrate | Lipolytic ratio LR(20/10) | References             |
|-------------------------|--------------------------|-----------|---------------------------|------------------------|
| GmsPLA₂-XIA-I           | 13                       | DLPC      | 0.45                      | This review            |
| GmsPLA₂-XIB-II          | 16                       | DLPC      | 0.25                      | This review            |
| B. diporus sPLA₂-I      | 11                       | DLPC      | ~0                        | Yunes Quartino et al., 2015 |
| B. diporus sPLA₂-II     | 12                       | DLPC      | ~0                        | Yunes Quartino et al., 2015 |
| M. fulvius-12           | 9–10                     | DLPC      | 0.07                      | Fernandez et al., 2017 |
| Pig pancreas PpsPLA₂    | 9                        | DLPC      | 0.08                      | Yunes Quartino et al., 2015 |
| Bee venom BvsPLA₂       | 18                       | DLPC      | 1.1                       | Yunes Quartino et al., 2015 |
| B. diporus BdsPLA₂-III  | 20                       | DLPC      | 1.3                       | Yunes Quartino et al., 2015 |
| B. asper BssPLA₂-III    | 18                       | DLPC      | 1.3                       | Yunes Quartino et al., 2015 |
| N. naja NmsPLA₂         | 17                       | DLPC      | 1.5                       | Yunes Quartino et al., 2015 |
| N. m. mossambica NmsPLA₂| 18                       | DLPC      | 1.6                       | Yunes Quartino et al., 2015 |
| M. fulvius-17           | 19–20                    | DLPC      | 1.7                       | Fernandez et al., 2017 |
substances and consume little energy (Warner, 2005). Natural and modified phospholipids have been extensively used in food industry, cosmetics, pharmaceuticals and agriculture (Guo et al., 2005). Therefore, in the production of these “modified phospholipids,” secreted phospholipases obtained mostly from microorganisms or mammals have been used by the industry either for refined oils, dairy products, baked goods and other health food industries (De Maria et al., 2007; Wang et al., 2012). As sPLA₂ enzyme catalyze the stereospecific hydrolysis at the chiral carbon (sn-2) of glycerophospholipids converting them to lysoderivatives, the enzymatic bioconversion is the only selective pathway for obtaining sn-2-lysophospholipids. Lysophospholipids have a greater bioemulsifiers capability and have been applied in food and pharmaceutical industries (Stafford and Dennis, 1988). In this regard, most sPLA₂s used are from animal pancreas (porcine or bovine) or venoms (bee, snake) since they are enzymes easily isolated in large quantities relatively to the low cost and they are commercially available (de Haas et al., 1968; De Maria et al., 2007). However, products of animal source, are rejected by many customers for religious reasons or risk of viral or prion contamination. Moreover, the use of enzymes from animal sources in processes for obtaining food additives may be incompatible with certain international regulations, which is not accepted in certain fields of application, since they do not meet the requirements of current international food standards. This is the reason why the industrial production of vegetable sPLA₂s may become desirable. Nevertheless microbial sPLA₂s are being accepted, sPLA₂s from plant would be an advantage because its putative natural specificity (Lee et al., 2005; Mansfeld, 2009).

In the last decade, research has focused on the study of the still little known vegetable sPLA₂s (Wang, 2001). Important advances have taken place in the identification, classification, biochemical characterization and functional analysis of plant sPLAs. Recent progress in understanding the biochemical and functional properties of plant sPLAs paves the way for approval of them for commercial use and various applications. Several sPLA₂s have shown great potential as a target in the field of plant biotechnology, and molecular and catalytic diversity of plant sPLA₂s shows that the phospholipases are of increasing value for biotechnology applications.

The possibility of using plant phospholipases in food processing would be an advantage, from the point of view of food regulations. Considering the large production of soybean in the world, it is of great interest to study the properties of its lipolytic enzymes in terms from of both agronomic and biotechnology point of views (Rönner, 2003; Hermida, 2005). Moreover, it should be noted that in the purification process of soybean oil, a byproduct named “gum” is a material enriched in phospholipids (about 65% of dry weight), which is usually used in animal’s food production or, after drying, it is sold as soybean lecithin. The hydrolytic products obtained by the action of sPLA₂ over soybean lecithin, the lysophospholipids (lysoderivatives), are widely used as emulsifiers (Henderson et al., 1995; Dashiel, 2001).

Recently, sPLA₂s were tested as catalysts for the synthesis of phospholipids with defined fatty acids by transesterification of lysophospholipids (Mansfeld, 2009). Furthermore, plant sPLA₂s showed to be distinctive from animals due to differences in substrate selectivity regarding the polar head and the acyl chains of glycerophospholipids (Lee et al., 2005). The potential properties of plant sPLA₂s would open new horizons to the engineering of biocatalysts.

The plant sPLA₂s is expected to have advantages over from animals regarding the performance or the incorporation of polyunsaturated fatty acids such as linoleic acid in egg PC for food production. Therefore, the processes for the production of phospholipids with fatty acids are not common and special performance requirements are desirable. Often, small differences in primary or 3D structure result in differences in the catalytic properties, which can be of great importance in biocatalytic applications. However, despite their enormous potential, plant enzymes have not been yet considered for industrial application. This could be attributed to the limited availability of these enzymes, recently discovered and characterized. Besides, these enzymes are much less abundant in the natural environment and no plant enzymes are available commercially.

Over 100 years, experiments with members of the sPLA₂ superfamily have been carried out and kinetic and structural characterization established sPLA₂ as an important model of interfacial enzymology. The future of this promising enzymes seems to be very exciting, leading to find out specific inhibitors of them, and further elucidating plants sPLA₂’s roles in cellular processes, along with potential uses in the industry.

AUTHOR CONTRIBUTIONS

MM and GF conceived the main idea, designed the general format of this manuscript, created the tables, and carried out the final corrections of this manuscript. MM prepared the figures and drafted this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.00861/full#supplementary-material
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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