Assessment of lipoxygenase activity in seeds and leaves of *Nigella sativa* and *Cassia absus*

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Background

Nigella sativa (family: Ranunculaceae), commonly known as black cumin and *Cassia absus* (family: Fabacea) commonly known as Chaksu are two important medicinal plants used in the treatments of many diseases.

The aim of the study was to investigate lipoxygenase (LOX) activity in seeds and leaves at different growth stages of these plants. Lipoxygenase LOX (linoleate oxygen oxidoreductase, EC 1.13.11.12) is an intramolecular dioxygenase catalyzing the hydroperoxidation of polyunsaturated fatty acids (PUFA) and forms two classes of products in plants (9(S) and 13(S)hydroperoxides which are precursors to a large amount of oxygenated derivatives that play an important role in the processes of plant ontogenesis and adaptation to stresses .

Nowadays, LOX have been found in a lot of varieties of plants, and is distributed in plant organs according to the type of environmental conditions, and the age of the plant [1], However, there is no information available about LOX in *N.sativa* and *C.abus*.

Methods

Plant growth conditions

N.sativa and *C.abus* were grown in the experimental greenhouse of the High Agronomic Institute of Chott-Mariem (Tunisia). Experiments were conducted in pots under normal crop conditions.

Culture period:

N. sativa (Indian variety) : from November to April

N. sativa (Tunisian variety) : from November to April

Seeds

Vegetative stage

Flowering stage

Fruiting stage

C.absus: from May to August

Cassia absus

Vegetative stage

LOX activity [2]

Preparation of the aqueous enzyme extract: Three gram of ground seeds or fresh leaves were extracted for 30 min at 0°C with 20 ml of acetate buffer (0.2M, pH 4,5). The extract was then centrifuged (3 000g , 20 min).

Enzyme assay:

LOX activity was determined spectrophotometrically by monitoring hydroperoxide increase at 234 nm. The inducible LOX activity was studied at pH7 using 20mM linolenic or linoleic acid as substrates. Experiments were conducted in triplicate.

❖ **Protein content determination** : Soluble protein concentrations were determined according to Bradford (1976)[3]. Experiments were conducted in triplicate.

Results

Lipoxygenase activity of seeds and leaves extracts from *N.sativa* (Indian variety)

N. sativa (Indian variety)	Protein content in the extract (%)	Substrate emulsion [20mM]	Activity µmole /g.s	Specific activity µmole /g protein.s
Seeds	1,8	Linolenic acid	0,88 (±0,22)	49,03 (±12,26)
		Linoleic acid	0,39 (±0,08)	21,73 (± 4,51)
Leaves (vegetative stage)	0,3	Linolenic acid	0,35 (±0,01)	106,47 (±3,36)
		Linoleic acid	0,08 (±0,00)	25,19 (±0,84)
Leaves (flowering stage)	0,4	Linolenic acid	0,35 (±0,01)	90,33 (±3,85)
		Linoleic acid	0,45 (±0,09)	116,15 (±23,38)
Leaves (fruiting stage)	0,3	Linolenic acid	0,38 (±0,11)	120,88 (±33,96)
		Linoleic acid	0,25 (±0,06)	78,16 (±20,09)

❖ Seeds of *N.sativa* exhibited the highest LOX activity [0,88 µmole/g.s)]. when the results are related to the protein content in the extract, leaves at fruiting stage of Indian variety show the highest level of biosynthetic capabilitie using linolenic acid as substrate.

Lipoxygenase activity of leaves extracts from *N.sativa* (Tunisian variety)

N. sativa Tunisian variety	Protein content in the extract (%)	Substrate emulsion [20mM]	Activity µmole /g.s	Specific activity µmole /g protein.s
Leaves (vegetative stage)	0,3	Linolenic acid	0,32 (±0,01)	94,6 (±2,36)
		Linoleic acid	0,1 (±0,03)	30,36 (±8,15)
Leaves (flowering stage)	0,3	Linolenic acid	0,3 (±0,02)	88,33 (±7,15)
		Linoleic acid	0,17 (±0,04)	52,83 (±12,97)
Leaves (fruiting stage)	0,2	Linolenic acid	0,41 (±0,06)	168,71 (±25,81)
		Linoleic acid	0,18 (±0,04)	74,73 (±15,08)

❖ Leaves at fruiting stage of Tunisian *N.sativa* variety exhibited the highest specific activity [168,71 µmole/g protein.s] in the presence of linolenic acid as substrate .

Lipoxygenase activity of seeds and leaves extracts from *C.absus*

C. abus	Protein content in the extract (%)	Substrate emulsion [20mM]	Activity µmole /g.s	Specific activity µmole /g protein.s
Seeds	0,2	Linolenic acid	0	0
		Linoleic acid	0,31 (±0,07)	181,68 (±41,32)
Leaves (vegetative stage)	0,3	Linolenic acid	0,46 (±0,03)	171,32 (±9,58)
		Linoleic acid	0,14 (±0,04)	50,17 (±15,71)

❖ *C.absus* seed LOX showed a high affinity for linoleic [181,68 µmole/g protein.s] rather than linolenic acid.

❖ *C.absus* leaf at vegetative stage showed high level of biosynthetic capabilities in the presence of linolenic acid as substrate [171,32 µmole /g protein.s].

Conclusion

❖ The results referring to the extracted protein content show that all extracts exhibited LOX activity.

❖ *N.sativa* LOX showed a preference for linolenic acid than linoleic acid. Leaves at fruiting stage of two *N.sativa* varieties exhibited the highest specific activity .

❖ *C.absus* seed LOX showed a high affinity for linoleic rather than linolenic acid. leaves at vegetative stage showed the highest level of biosynthetic capabilities using linolenic acid as substrate .

❖ In conclusion, this study shows that LOX is involved in growth and development of *Nigella sativa* and *Cassia absus* especially in fruit ripening.

References

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