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RESEARCH ARTICLE

Alterations in the primary metabolite profiles of field dodder (*Cuscuta campestris*) and its associated hosts cutleaf evening primrose (*Oenothera laciniata*) and swine cress (*Coronopus didymus*) in the fields of North-West India

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ABSTRACT

Cutleaf evening primrose (*Oenothera laciniata* Hill.) and swine cress (*Coronopus didymus* (L.) Sm.) are the two weeds which were found to be infested by a stem holoparasitic plant, field dodder (*Cuscuta campestris* Yunck.) in the fields of Punjab Agricultural University, Ludhiana, Punjab, during 2023-24. Although, detailed records of host-parasite associations of *Cuscuta* spp. are present in a large number of field crops predominantly belonging to families Solanaceae, Poaceae, Leguminosae and Brassicaceae; this is the first report to our knowledge of parasitic associations of *C. campestris* to two new weeds as hosts – *O. laciniata* and *C. didymus* belonging to the families Onagraceae and Brassicaceae, respectively in the fields under continuous rice-wheat rotation since more than twenty years. Parasitic plants are finding new hosts owing to increasing concerns of habitat suitability and host variability amidst rising trends of global climate change for agriculturalists. Biochemical analysis documented nutrient acquisition by field dodder from weed hosts, demonstrating the different host parasite assemblages as cause for variation in the primary metabolites' profiling in the hosts as well as the parasite. Evidently, the stem and leaves of *O. laciniata* and *C. didymus* were used by this noxious parasitic weed as a means for reaching the fruits of its host for maximum nutrient acquisition so as to complete its life cycle and expanding its seed bank in a field where its few seeds may have accidentally arrived from an unknown source.

Keywords: *Cuscuta campestris*, Haustoria, Host, Parasite, Proteins, Starch, Total soluble sugars

INTRODUCTION

Total parasitic angiosperms are non-photosynthesising plants which are totally dependent on the host plant for photosynthates as well as water and nutrients. They span over 12 families comprising of about 300 genera grouping more than 4500 species (Nickrent *et al.* 2020). Regardless of the taxonomical classification or degree of parasitism, they all share a common feature called the haustorium (pl: haustoria) (Albanova *et al.* 2023). Haustoria are the functional physiological links between hosts and parasites through which they acquire solutes, nutrients, minerals and carbohydrates from their hosts, but also bidirectionally exchange signalling molecules and pathogens (Kim and Westwood 2015). Despite the parasitic nature, very few such plant species represent relatively damaging agricultural pests' responsible for annual yield losses and ecological threats. The most damaging parasitic plants belong to the *Cuscuta* spp. from the Convolvulaceae family and *Striga* spp. and *Orobanche* spp. from the Orobanchaceae family (Kaiser *et al.* 2015).

Parasitic plants of the genus *Cuscuta* are referred to as cryptically photosynthetic due to the absence of or negligible levels of chlorophyll, making them non-photosynthetic. Thus, all *Cuscuta* spp. (commonly known as dodder) are obligate holoparasites dependent on the host plant, mostly herbs and shrubs to complete their life cycle since a germinating *Cuscuta* seedling has very limited seed reserves (Patel and Patel 2010). It neither bears root nor fully expanded leaves and the only vegetative portion appears to be the thin pale stem, thus it must attach itself to an appropriate host plant with in the initial days. *Cuscuta* spp. recognize and infests the host plant *via* releasing chemoattractant plant volatiles directing the successful parasitic growth and infection (Kaiser *et al.* 2015).

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Cuscuta spp. have become a major problem in many field crops, *viz*. berseem, lucerne, tomato, potato, mustard, soybean, chillies, chick pea, green gram, black gram, lentil, alfalfa, onion, *etc*. (Albert *et al*. 2008). Huge crop losses have been reported due to *Cuscuta* in 25 crop species in 55 countries (Lanini and Kogan 2005). The yield reductions due to infestation of *Cuscuta* spp. have been reported to be 60–65% in chillies, 31–34% in green gram/black gram, 87% in lentil, 86% in chickpea, 72% in tomato and 60–70% in alfalfa (Mishra 2009). This parasitic plant establishes physiological connection with the host plant through haustoria for penetrating the vascular bundles of the host to withdraw water, carbohydrates and other resources.

Oenothera laciniata (commonly known as cutleaf evening primrose) belongs to the family Onagraceae. It is an annual or short-lived perennial herb producing a spreading stem. Leaves are lanceolate, deeply notched, medium green with hairs on the surface and none below. Flowers occur in the axils of leaves higher on the stem. Each flower has pale to deep yellow petals which fade orange, pink, or red with age. The fruit is a cylindrical capsule up to 5 centimeters in length. *O. laciniata* is endemic to the eastern United States but has been reported from many other countries including India as a noxious weed in agriculture. It has also been reported from different countries such as Hawaii, Australia, Britain, France, Korea and Japan (Nayar *et al*. 2012).

Coronopus didymus (commonly known as swine cress) is a small herbaceous annual weed belonging to the family Brassicaceae growing in rosette on ground with a 15-30 cm long prostate stem, commonly seen growing in pasture, wastelands and along roadsides between an altitude of 700-3000 m in Western Himalaya. Its seeds are easily disseminated by wind and are harvested for food and medicine. Leaves are pinnatifid or pinnatipartite with lobes spreading, almost entire. Flowers are small pale green with sub-erect sepals and short or no petals, 1-2 mm in size situated in elongated, lateral racemes. Fruits are bilobed 1.5 to 2.5 mm in size producing falcate, finely netted and brown seeds (Anonymous 2019).

The association of *Cuscuta* spp. has been reported with many field crops (Holm *et al*. 1997). In this paper, we report the first observation on the host-parasite interactions of *C. campestris* with two weeds namely *O. laciniata* and *C. didymus* along with the alterations caused in the profile of primary metabolites of the associated parasite as well as host plants.

MATERIALS AND METHODS

The host-parasite assemblage of *C. campestris* with *O. laciniata* and *C. didymus* has been observed since many years during the months of April and May on a five-acre field in Punjab Agricultural University, Ludhiana, Punjab, India (30°56'N latitude, 75°52'E longitude and at an altitude of 247m msl). This site represents the Indo-Gangetic alluvial plains with a sub-tropical, semi-arid climate with characteristically hot summers and very cold winters. The soil was loamy sand and rice-wheat rotation was followed on the infested area from last many years and has been irrigated with canal water.

Samples from the parasite *C. campestris* and infected host plants were collected for biochemical investigations. Infected and non-infected leaves – lamina and petiole, stem and fruits were collected from the hosts alongside the respective *Cuscuta* stem, haustoria and flower. These samples were used to analyze various primary metabolites, *viz.* sucrose, total soluble sugars, starch, total soluble proteins and total free amino acids. Total soluble sugar content was estimated from the ethanolic extractions *via* phenol-sulphuric acid method as stated by Dubois *et al.* (1956). Starch was extracted in the form of soluble sugars from the residue left after the extraction of total soluble sugars using perchloric acid as described by Clegg (1956). The extracted sugars were similarly estimated *via* the phenol-sulphuric acid method (Dubois *et al.* 1956), and the starch content was calculated by multiplying the content of total soluble sugars obtained in the residue by a factor of 0.9.

Sucrose content was estimated using the ethanolic extraction as per the standard method stated by Roe *et al.* (1949). The extract was evaporated to dryness at 100°C in a water bath and the volume was raised upto 10ml with distilled water. To this, 1ml of saturated lead acetate solution was added and kept overnight for the proteins to precipitate. The extracts were filtered and a pinch of sodium oxalate was added to the clear supernatants. The extracts were filtered again following an overnight incubation to obtain a clear extract. A reaction mixture prepared by adding 0.5ml extract and 0.5ml 6% KOH was heated in a boiling water bath for 20 minutes, following which 1ml of resorcinol reagent and 3ml of 30% HCL were added and the tubes were again incubated in a boiling water bath for 20 minutes. The pink coloured complex developed was read at 490 nm on a UV-Visible Spectrophotometer (Systronics UV-VIS Spectrophotometer 117).

For extraction of total soluble proteins and total free amino acids, 0.1g tissue sample was extracted in 10ml of 0.1N sodium hydroxide (NaOH). 2ml of protein extract was further taken in new tubes, adding 2ml of 20% trichloroacetic acid to the tubes and incubating at 4°C for 24 hours. The precipitates obtained were dissolved in 0.1N NaOH. This extract was used to estimate total soluble protein content as per the estimation procedure stated by Lowry *et al.* (1951). A reaction mixture of 0.1ml extract, 0.4ml distilled water, 2.5ml solution prepared by mixing 2% sodium carbonate in 0.1N NaOH and 0.5% copper sulphate in 1% potassium sodium tartarate in ratio 50:1 and 0.25ml folin-ciocalteau reagent diluted with distilled water in 1:1 ratio. The blue coloured complex formed was read at 520nm against a reagent blank following incubation in the dark for 60 minutes. Total free amino acids were estimated as per the protocol of Lee and Takahashi (1966). A reaction mixture of 0.2 ml extract, 0.8ml distilled water and 4ml ninhydrin reagent was incubated in a water bath at 90°C for 1 hour. The violet coloured complex developed was read at 570nm on a UV-Visible Spectrophotometer after cooling the tubes against a reagent blank.

The analysis of variance for primary metabolites was computed using the Minitab (2017) software. The ANOVA was analyzed for variation in host-parasite primary metabolite profile using Tukey's test.

RESULTS AND DISCUSSION

C. campestris thrived luxuriantly on the *O. laciniata* fruits with few leaves also penetrated by this parasite. On an average 10-15 haustoria of *Cuscuta* penetrated the fruits of *O. laciniata*. Stem and leaves of *O. laciniata* were mostly used by *C. campestris* as means for reaching to another fruits. Abundant seed production was observed in host as well as parasite. Due to inability of this weed to parasitize cereals like rice and wheat, this parasitic weed must have infested these weeds for completing its life cycle for increasing its seed bank in a field where its few seeds might have landed accidentally. These new host-parasite assemblages could be attributed to the continuous rice-wheat rotation in Punjab, India combined with the ecological perturbations led by global climate change. These climatic changes have worsened the issue of invasive alien plants and weeds in the agro-ecosystems at a global scale resulting in altered parasite transmission, range changes and population densities, increasing the potential for host switching (Brooks and Hoberg 2007).

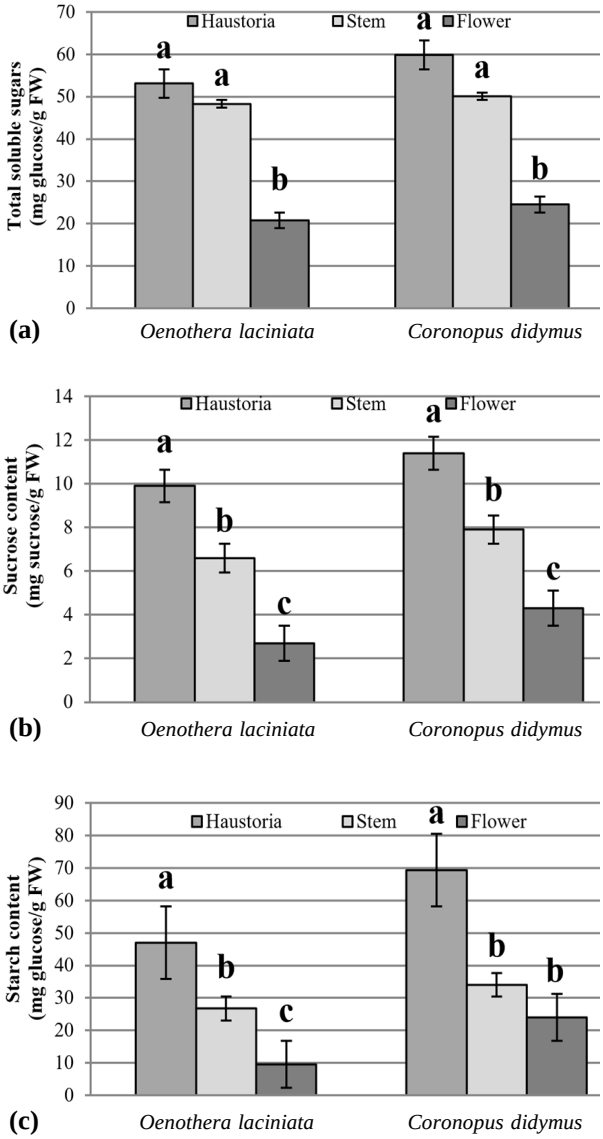

Figure 1. (a) Total soluble sugars, (b) sucrose and (c) starch content of *Cuscuta campestris* Yunck. Parasitizing on the two host plant species, *Oenothera laciniata* Hill and *Coronopus didymus* (L.) Sm. Each bar represents mean ± standard error. Least square means with different superscript letters are significantly different

Biochemical analysis of the *C. campestris* penetrating two different hosts revealed variation in the metabolite profile of three organs – haustoria, stem and flowers. Haustoria being the specialized intrusive organ for water and nutrient absorption, recorded the highest contents of primary metabolites, *viz*. total soluble proteins, total soluble sugars, sucrose and starch, followed by stem and then flowers (**Figure 1** and **2**). *C. chinensis* has also been recorded to divert huge amount of nitrogen and carbon from the host plants, completing its own life

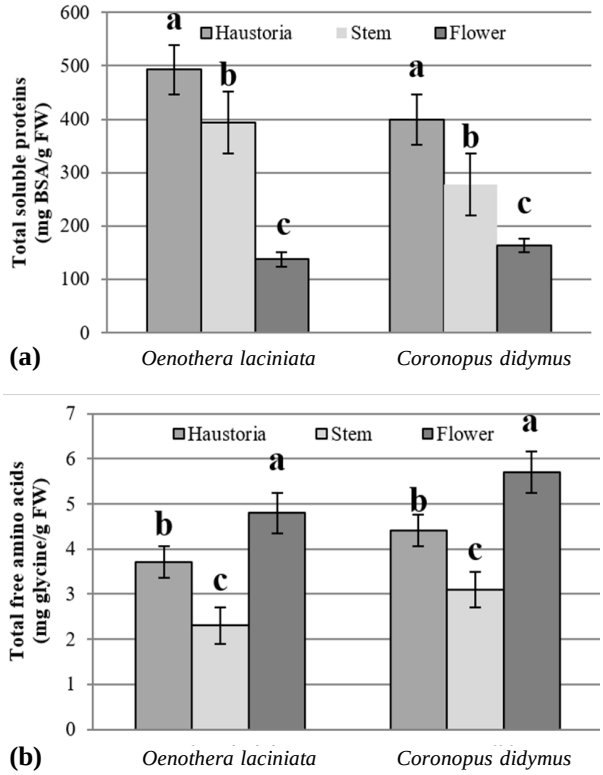

Figure 2. (a) Total soluble protein content and (b) total free amino acid content of *Cuscuta campestris* Yunck. parasitizing on the two host plant species, *Oenothera laciniata* Hill and *Coronopus didymus* (L.) Sm. Each bar represents mean ± standard error. Least square means with different superscript letters are significantly different

cycle at the cost of suppressing the host growth and development (Marambe *et al*. 2002). Comparatively, *C. campestris* extracted higher carbohydrate reserves from the host *C. didymus* than *O. laciniata* (**Figure 1**). Thus, difference in the host also contributes to alteration in the metabolite composition of the parasite. The alterations in the metabolite profile following parasitization are a complex issue. Furthermore, it is extremely challenging to distinguish the host-derived or parasite created metabolites. Amino acids recorded an atypical trend than the other metabolites, with flowers recording the highest total free amino acids followed by haustoria and stem (**Figure 2b**). Many differential results on the altered amino acid composition have been perused in the literature as the free amino acid composition fraction is exceedingly susceptible to the variations in the environmental conditions and the host plant (Borghi *et al*. 2017). Amino acids are in addition also an important constituent of floral secondary metabolism following similar partitioning routes to sugars and aiding in *de novo* amino acid synthesis in floral tissues forming a completely different amino acid profile than haustoria and stem which is further used as a nitrogen storage compound for energy for a high C:N ratio. This higher C:N ratio contributes to energy generation for ovule and pollen development, successful fertilization, maturation and embryo growth (Tsai and Chang 2022). *De novo* synthesis of free amino acids like proline, asparagine and valine is also particular to floral tissues forming protein component of pollen coat, scent, colour and nectar for pollinators (Borghi *et al*. 2017). This also allows us to assume the increasing proclivity of *C. campestris* towards seed formation in the studied host-parasite association.The investigation results documented a significant impact of the parasitic weed on the primary metabolites of the host plant. Also, a significant difference was recorded in the parasitic metabolic profile depending on the different host they developed on (**Figure 1** and **2**), suggesting the high reliability of the parasites on the host's metabolites for nutrient acquisition. Our results were in accordance with the findings of Kumar and Amir (2021). They similarly reported notable variation in the *Cuscuta campestris* metabolic profiles that developed on the different hosts, indicating that the parasites' were heavily dependent on the host plant for metabolites. However, ample

Table 1. Primary metabolite profile, *viz*. total soluble proteins, free amino acids, total soluble sugars, starch and sucrose content in *C. campestris* infected and non-infected host plant – *O. laciniata*

Oenothera laciniata Hill	Total soluble protein content (mg BSA/g fresh weight)		Total free amino acid content (mg glycine/g fresh weight)		Total soluble sugar content (mg glucose/g fresh weight)		Total starch content (mg glucose/g fresh weight)		Total sucrose content (mg sucrose/g fresh weight)	
	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected
Lamina	76.9^{b}±1.3	210.3^{a}±2.2	2.4^{a}±0.05	1.4^{b}±0.03	6.0^{b}±0.4	12.5^{a}±0.2	14.6^{b}±0.8	28.5^{a}±2.4	1.8^{b}±0.12	2.4^{a}±0.08
Petiole	63.1^{b}±0.6	80.9^{a}±1.3	3.1^{a}±0.09	2.8^{b}±0.06	4.5^{b}±0.9	9.7^{a}±0.9	15.5^{b}±1.2	23.5^{a}±0.1	1.3^{b}±0.03	2.0^{a}±0.10
Stem	60.7^{b}±1.8	73.0^{a}±0.6	3.8^{a}±0.01	2.3^{b}±0.06	10.4^{b}±0.3	11.5^{a}±0.2	25.7^{b}±0.2	33.9^{a}±0.6	2.3^{b}±0.12	3.7^{a}±0.08
Fruit	252.6^{b}±4.2	540.7^{a}±30.1	3.1^{a}±0.34	1.5^{b}±0.07	6.7^{b}±0.2	23.9^{a}±0.9	24.4^{b}±2.1	60.5^{a}±2.5	0.3^{b}±0.08	4.7^{a}±0.26

All results are expressed as mean ± standard error at p=00.5

Table 2. Primary metabolite profile, *viz*. total soluble proteins, free amino acids, total soluble sugars, starch and sucrose content in *C. campestris* infected and non-infected host plant – *C. didymus*

Coronopus didymus (L.) Sm.	Total soluble protein content (mg BSA/g fresh weight)		Total free amino acid content (mg glycine/g fresh weight)		Total soluble sugar content (mg glucose/g fresh weight)		Total starch content (mg glucose/g fresh weight)		Total sucrose content (mg sucrose/g fresh weight)	
	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected	Infected	Non-Infected
Lamina	201.3^{b}±1.3	244.5^{a}±0.6	4.9^{a}±0.02	2.9^{b}±0.12	11.0^{b}±0.4	36.8^{a}±1.4	20.9^{b}±1.9	29.5^{a}±0.8	3.7^{b}±0.38	15.4^{a}±0.26
Petiole	124.3^{b}±1.6	184.9^{a}±0.6	5.5^{a}±0.06	2.8^{b}±0.08	12.6^{b}±0.1	32.8^{a}±0.2	20.8^{b}±0.1	22.2^{a}±0.4	4.2^{b}±0.38	11.6^{a}±0.54
Stem	83.8^{b}±2.9	124.5^{a}±0.6	3.1^{a}±0.06	1.6^{b}±0.04	6.6^{b}±0.4	25.4^{a}±0.2	10.8^{b}±0.7	35.9^{a}±1.1	2.6^{b}±0.03	6.8^{a}±0.21

All results are expressed as mean ± standard error at p=00.5

results were also obtained which infer that the parasite can self-regulate its metabolic profile between organs *via* anabolic-catabolic activities. Similar to our results, Kumar and Amir (2021) also reported higher amino acids and sugar acids in the flowers, with significantly higher levels of most sugars and polyols in the haustoria and stem.

C. campestris penetrating *O. laciniata* recorded pronounced variation in the primary metabolite profile in the host organs – lamina, petiole, stem and fruit (**Table 1**). The highest content of nutrients was extracted from the fruit with a decrease of 72% in total soluble sugars, 93.6% in total sucrose, 59.7% in starch and 53.3% in total soluble proteins with a 51.6% increase in the total free amino acids. This can be ascribed to enhanced phloem unloading at the site of attachment of *C. campestris*. **Table 2** tabulates the variation in the primary metabolite reserves of *C. didymus* following the parasitic infestation of *C. campestris* on the host weed. Host-parasitic assemblage was formed on three host organs – lamina, petiole and stem. A significant decrease of 70.1%, 76% and 26.2% was recorded in total soluble sugars, sucrose and starch content, respectively in the infected *C. didymus* plant lamina than non-infected. Significant increase of 40.8%, 49.1% and 48.4% was recorded in total free amino acid content in infected lamina, petiole and stem of the *C. didymus*, respectively. This increase in the free amino acids was parallel to the 17.7%, 32.8% and 32.7% decrease in the total soluble proteins in lamina, petiole and stem in the infected host respectively. *Cuscuta* can withdraw almost all photosynthates originally intended for the developing host fruits *via* an unusually enhanced phloem unloading rate as recorded in *Vicia faba* beans (Wolswinkel *et al*. 1984). This could be attributed to the fact that primary metabolites such as amino acids and carbohydrates are energy sources and precursors of floral secondary metabolism and seed set (Borghi *et al*. 2017).

Conclusion

The stem and leaves of *O. laciniata* and *C. didymus* were used by *C. campestris*, a noxious parasitic weed as a means for reaching the fruits of its host for nutrient acquisition so as to complete its life cycle and expanding its seed bank in a field where its few seeds may have accidentally arrived from an unknown source.

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