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Cover Photo

Top left: Pistil of *Oryza sativa*, **Top right**: T.S of wild rice (*Oryza rufipogon*) stem, **Bottom left**: Seeds of *Vigna unguiculata* subsp. s*esquipedalis*, **Bottom right**: schematic representation of zinc oxide nanoparticles.

Technical Assistance: Dr. Swarnendu Roy, Ashis Sarkar

EDITORIAL

The NBU Journal of Plant Sciences (NBUJPS) started its journey in the year 2007 and has been publishing quality articles in print mode. It is my honour to inform that the Editorial Board has decided to publish the journal in both print and online mode from 2021 (starting Volume 13, March 2021). From now, all the articles of the present and past volumes can be accessed at www.njps.nbu.ac.in. The NBUJPS have officially registered for the membership of CrossRef and now onwards all the articles will be assigned unique DOI numbers and will include other CrossRef services like similarity checking and reference linking. It gives me extreme pleasure to inform that the NBUJPS publishes all the articles at free of charge and will continue to do so in the future also.

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Research Article

Study on Spikelet Morphology of Some Indian Grasses (Poaceae)

Sujit Mondal, Aaratrik Pal and Monoranjan Chowdhury*

Taxonomy of Angiosperms and Biosystematics Laboratory, Department of Botany, University of North Bengal, Siliguri–734 013, Darjeeling, West Bengal, India

Abstract

Poaceae (Graminae) is one of the largest vascular flowering plant family that includes many economically valuable food crops that cultivated in different parts of the World. Grasses are most common floral component of all kind of vegetation and they grow in marshy lowland to higher altitudes. Identification at the species level becomes extremely difficult for grasses and only the revelation of detailed spikelet features can be a strong tool to provide a satisfactory result. Present work studied detailed morphology of various parts of spikelet of some grass from sub-Himalayan West Bengal along with their suitable illustrations.

Keywords: Spikelet, Glumes, lodicule, Poaceae

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Introduction

The grass family, Poaceae is the fourth largest angiosperm family having about 12000 species under 780 genera (Watson & Dallwitz, 1992; Clayton & Renvoize, 1986; Christenhusz & Byng, 2016) which are economically, ecologically, and evolutionarily successful species rich groups in the world (Calderon & Thomas, 1973). In India Poaceae is the largest family which comprises about 1300 species belonging to 268 genera (Karthikeyan *et al.*, 1989; Moulik, 1997). About 430 grass species belongs to 15 genera are strictly endemic to India and 40% of them are concentrated in Peninsular India (Jain, 1986). There are two are monotypic genera and species of 13 genera are strictly restricted to Peninsular India (Kiran Raj *et al.* 2003). Since the dawn of human civilization, the value of the grasses to the mankind has been recognised. The members of this family are present in all the suitable habitats for their growth (Mitra & Mukherjee, 2015). Noltie (1994) described 18 genera with several species which provided us a broad idea about Poaceae from Eastern Himalayan region. But the spikelet structure is not previously described in-detail. Prain (1903) described 405 species with 133 genera from undivided West Bengal. Pal *et al.* (1991) and Pal (2010) described 34 species of Bengal's grasses in his book "*Banglar Ghas o Bash*''. This book plays an instrumental role in the identification of the family Poaceae.

Materials and Methods

A thorough survey of the Terai Duars areas was made during mid-February to mid-June for the collection of grasses at their flowering stage (2018-2020). The specimens were collected and temporarily preserved in large polythene bags with mouths being kept tied to prevent the desiccation. After return to the laboratory, the specimens were cleaned and pressed within old newspaper under heavy wooden herbarium press. After proper drying of specimens were poisoned and mounted on herbarium sheets to prepared voucher specimens (Paul et al., 2020). Identification of all the collected specimens was done in the Taxonomy of Angiosperms and Biosystematics Lab., Department of Botany, North Bengal University using available literature and matching with the available predetermined specimen in the NBU Herbarium and voucher specimens were deposited in NBU herbarium. Fresh spikelet of all the collected specimens were dissected and observed under microscope critically. Each part of spikelet was drawn and measurement also recorded.

* ***Correspondence*** - mono_malda@yahoo.co.in

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Result and Discussion

Spikelet morph-taxonomical study on commonly available grass species was done. All the 14 selected species are representative of six different tribes (table 1). The parts of spikelets and their peculiarities are clearly showing in Fig 1 and 2. The studied morphological feature are quite useful for the definite identification of grass species and for easy identification a genus key is provided.

Key to the genera

1a. Cultivated, annual 2

1b. Wild, annual or perennial 3

2a. Glume 1 & 2 reduced to minute swellings, spikelet sometimes subtended by glume like sterile floret, palea l-keeled ... .*Oryza*

2b. Glume 1 & 2 not reduced to minute, inflorescence spike like, glume hairy .... *Triticum*

3a. Spikelets arranged in racemes 4

3b. Spikelets not arranged in racemes 7

4a. Racemes arranged in 1 row ....*Axonopus*

4b. Racemes arranged in more than I row 5

5a. Raceme usually in 3 or more rows, with spikelet inserted singly or in pairs either side of rachis midrib ....*Paspalum*

5b. Spikelets not inserted singly or in pairs either side of rachis midrib 6

6a. Raceme narrow; grain oblong narrowly lanceolate: palea narrowly winged ....*Eleusine*

6b. Racemes usually under 2 cm ....*Echinochola*

7a. Glumes long-aristate bristle-like, sticky or hispid..... ....*Oplismenus*

7b. Glumes not long-aristate bristle-like..... 8

8a. Spikelet whitish or flushed purplish-pink ....*Eragrostis*

8b. Spikelet not whitish or flushed purplish-pink 9

9a. Spikelet golden to reddish brown below, glabrous ....*Saccharum*

9b. Spikelet not whitish flushed or purplish-pink 10

10a. Spikelets with awn ....*Chrysopogon*

10b. Spikelet without awn 11

11a. Floret single .... Cy*nodon*

11b. Florets many 12

12a. Hairs from callus or back to the glume ....*Imperata*

12b. Lateral veins of lower lemma lacking spicules near apex ....*Digitaria*

Table 1 Collected grasses are belonging to the six tribes of Gramineae

Tribe	Taxa
Paniceae	*Axonopus compressus. Digitaria ciliaris, Echinochloa colona, Oplismenus burmannii, Oplismenus compositus, Paspalum scrobiculateum*
Andropogoneae	*Chrysopogon acciculatum, Impereta cylindrica, saccharum spontanum*
Cynodonteae	*Cynodon dactylon*
Eragrostideae	*Eleusine indica, Eragrostis uniloides*
Oryzeae	*Oryza sativa*
Triticeae	*Triticum aestivum*

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Axonopus compressus (Swatz) P. Beauvois, Ess. Agrost. 12, 154. 1678. Bor Grass. Ind 27. 1960. *Millius compresus* Sw., Prodr. Veg. Ind. Oec. 24. 1788; Noltie, Fl. Bhutan 3(2):717. 2000. **"*Choto chepti (Beng)".***

Spikelets 2–2.5 mm. Glumes 2–2.5 × 0.8–1 mm, oblong, acuminate, back flat, veins appressed hairy, with long, wooly hairs on incurved sides below and at truncate base: lower lemma 18–2.2 × 0.7–09 mm, oblong lanceolate, acuminate to apiculate, back 4 veined, flat margins incurved: upper lemma pale green, 1.6–1.7 × 0.8–1 mm. compressed, oblong-elliptic, blunt, with apical cilia, crustaceous palea 1.5–1.6 × 0.7–0.8 mm, similar to palea, but glabrous, lodicules 2; stamens 3. Styles 2, free: stigmas plumose.

Specimen cited: Mahananda Wild Life Sanctuary (MWLS), Mondal *et al.* 006. 23.04.2019

Chrysopogon aciculatus (Retzius) Trinius, Fund. Agrost. 188. 182; Ohwi in Act. Phytotaxa Geobot. 11: 162. 1942. *Andropogon aciculatus* Retz., obs. Bot. 5. 1789: Hook. *f.* Fl. Brit. Ind. 7: 188. 1986; Prain, Beng Pl. 2. 1205. 1903. Noltie, Fl. Bhutan 3(2):791. 2000.

Sessile spikelet 31–4 mm; callus short hairs golden the longest 0.5–0.9 mm: glume membranous, the lower 3.1–3.8 0.7 mm, narrowly oblong lanceolate, apex bidenticulate back not veined, keels tuberculate - hispid above: upper glume narrowly lanceolate, apex mucronulate keel minutely ciliate above margins widely hyaline; lower lemma 2.5–3mm. linear-lanceolate, acute; palea 16–1.8 mm. oblong, rounded often absent: upper lemmu2.2–2.9 mm, awn 3.5–57 mm; anthers 1.4 mm, spikelets subequal, 4.6–5.7 mm: lower glume 4.5–5.7 mm, narrowly oblong lanceolate. acuminate, midrib minutely hispid above, keels smooth upper glume 3.8–4.7 mm, narrowly oblong. minutely apiculate, margins ciliate: palea 1.4–2.1 mm, linear, acute; upper lemma similar to lower; anthers 2 mm pedicels subequal, 2–3.8 mm more face concave, glabrous. Caryopsis linear 0.2–0.4 mm long

Specimen cited: Mahananda Wild Life Sanctuary (MWLS), Mondal *et. al.* 001 dated 17.03.2019.

Cynodon dectylon (Linnaeus) Persoon, Syn. Pl. 1: 85. 1805; Hooker *f.*, Fl. Brit. Ind. 7: 288. 1896; Bor., Grass. Ind. 469, f. 52.1960; Noltie, Fl. Bhutan 3(2): 678 2000. *Panicum dactylon* L., Sp. Pl. 58. 1753. ***"Durba ghas"***

Spikelets 1.9–2.7 mm. Lower glume 1–19 mm. subacute, each half 0.2–0.3 mm, keel

minutely serrate: upper glume 1–2.2, each half 0.2–0.3 mm wide, lemma 1.7–25 mm, each half semi-lanceolate, acute, 0.6–0 .8 mm wide, keel ciliate, stopping just below apex, Palea 15–2×03–0.5 mm; anthers 1.1 mm. Vestigial rachilla 0.5–1 .2 mm, so sometimes slightly widened at apex. Stamens 3, Caryopsis oblong + 0.15 cm long brown

Specimen cited: Buxa Tiger Reserve. Mondal *et. al.* 22. dated 12.04.2019

Digitaria ciliaris (Retzius) Koeler, Deser Gram, 27. 1802; Noltie, Fl Bhutan 3(2):728. 2000. *Panicum ciliare* Retzius, Obs. Bot 4: 16 1786. *Digitaria sanguinalis* Scopoli var. *ciliaris* sensu Prain, Beng Pl. 2:1181, 1903.

Inflorescence axis 0.8–13 cm. Racemes 3–6, digitate or lower 2–3 slightly distant, the lowest 25–10 cm; rachis flattened winged, margins hispid. Spikelets paired, unequal pedicellate. 2.8–3.4 × 0.8–1 mm, lanceolate, acute. Lower glume small, 0.25–0.4 mm, triangular, glabrous: upper glume 1.5–1.8 0.4–0.5 mm, lanceolate, acute, 3-veined, margins fong - ciliate. Lower lemma equaling spikelet, 2.8–3.4 × 08 – 1 mm. lanceolate, acuminate, 5 veined, outer 2 pairs close to margins, inter nerve space next to midrib broad, appressed long-hairy between outer veins. upper floret lemma cream-coloured. 2.6–31 mm, narrowly oblong lanceolate, acuminate: palea 2,5–3 mm: anthers 0.9 mm. Stamens 3. Caryopsis +0.2 cm long.

Specimen cited: Buxa Tiger Reserve, Mondal *et. al*. 03, dated 21.03. 2020.

Echinochola colona (Linnaeus) Link, Enum. Hort: Berol. 2: 209. 1833. Haines, Bot. Bihar Or. Pr 5: 997. 1924, Bor, Grass. Cey. Ind and Pak. 308,1960. Noltie, Fl. Bhutan 3(2).702. 2000. *Panicum colonum* U. Syst. Nat. Ed. 10(2): 870. 1759. Hook. *f.*, Fl. Brit. Ind. 7:32.1896; Prain, Beng Pl. 2:1177.1903.

Inflorescence 4.5-11 cm; raceme suberect, all except uppermost rather distant, the lowest 12.2 cm, axis straight, minutely hispid, sometimes also with long cilia. Spikelet 25–3mm. Lower 14–15 mm. ovate, acuminate, shortly cuspidate, 5–8 veined Lower floret lemma 22–29 mm, oval, acuminate, 7-veined; palace 1.8–22 × 0.8–1 mm, oblong-elliptic. Upper floret lemma 19–24 × 1–15 mm, narrowly elliptic, apiculate 0.2 mm, polycab 16–2 × 1.3 mm, anthers 0.8 mm.

Specimen cited: NBU campus. Mondal *et al* dated 18.03.2020.

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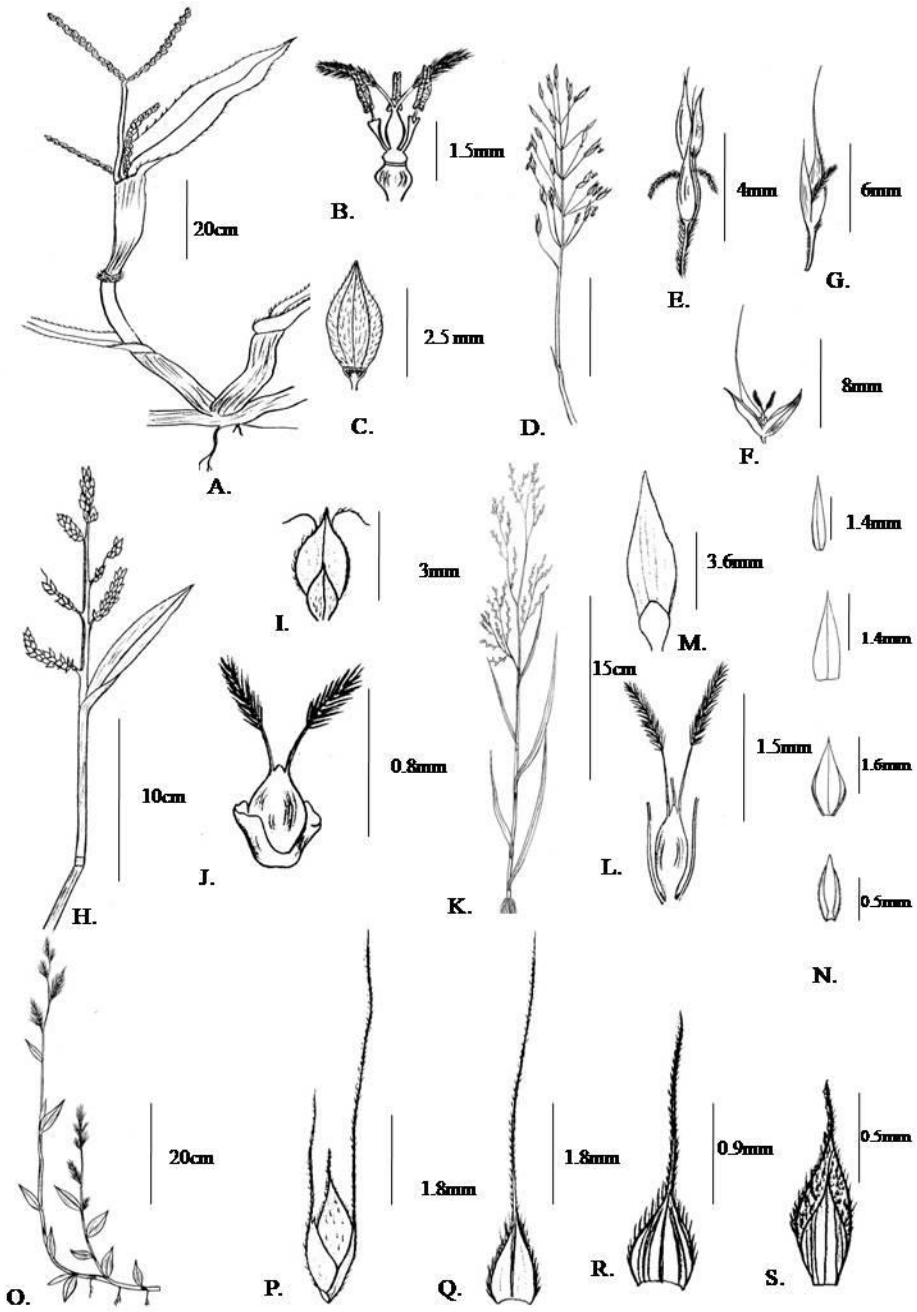

Fig. 1: A-C. *Axonopus compessus*, **A.** habit, **B. s**tamen and pistil **C.** spikelet; **D-G.** *Cymbopogon pendulus*, **D.** habit, **E.** spikelet, **F & G.** female flower; **H-J.** *Echinocloa colona*, **H.** habit, **I.** spikelet, **J.** pistil; **K-N.** *Eragrostis uniloides*, **K.** habit, **L.** pistil, **M.** spikelet, **N.** glumes; **O-S.** *Oplismenus burmannii*, **O.** habit, **P.** spikelet, **Q.** lower glume, **R.** upper glume, **S.** lower lemma.

Eragrostis unioloides (Retzius) Nees *ex* Steudel, Syn. Pl. Glum. 264. 1854; Owin, Bot. Mag. Tokyo 55: 275. 1941; Noltie, Fl. Bhutan 3(2): 663. 2000. *Poa unioloides* Retz., Obs. Bot. 5: 19. 1789.

Inflorescence 3.5–20 × 1–6 cm. cylindric, moderately dense. branches single, ascending, aglandular, under 10 mm. Spikelets whitish purplish-pink 52–14 × 23–32 mm, lateral veins of lemmas raised so spikelets rather flat in cross section, florets 19–69, glumes, lemmas and palea deciduous from base upwards, Lower glume l.4–1.6 × 0.4–0.5 mm lanceolate, acuminate, upper glume 1.4–22 × 0.6–0.8 mm, oblong-lanceolate, subacute Lower floret: lemma 1.4–2.3 × 0.7–1.2 mm, oblong lanceolate ovate, shortly acuminate, surface papillose, lateral veins conspicuously raised, palea 14–18 × 0.6–0.9 mm, narrowly elliptic, truncate keels hispid; anthers 0.2–0.5 mm: grains 0.6–0.7 × 0.3 – 0 .5 mm, oblong-elliptic. smooth, slightly compressed in sections.

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Specimen cited: Gurumara National Park. Mondal *et al* 07 dated 03.04.2019.

Elusine indica (Linnaeus) Gaertner, Fruct. 1: 8. 1789; Hook. *f*., FI. Brit Ind. 7: 293. 1896: Ohwi, Bot. Mag. Tokyo 55: 312. 1941: Bor, Grass. Ind. 495. 1960; Noltie, Fl Bhutan 3(2):667-668. 2000. *Cynosurus indicus* L., Sp. Pl. 72 1753.

Spikelets 2–5 mm long, 3–6 flowered, densely imbricate in 2 rows, pointing upward at an acute angle with the rachis. Glumes membranous, unequal: lower oblong-ovate or oblong, subacute, the lowest 3–3.6 mm long, palea not winged on the keels. Caryopsis oblong, obtuse trigonous.

Specimen cited: NBU campus, Mondal *et. al.* 06 dated 01.04.2020

Imperata cylindrica (L.) Rausch., Nom. Bot ed. 3: 10. 1797, Bor, Grass 169 1960: Recder, Tourn. Arm. Arb. 29: 327. 1948; Hara, Fl. E Himl. 366.1966; Noltie, Fl. Bhutan 3(2):770 – 771. 2000. *Lagurus cylindericus* L., Syst. Nat. Ed. 10 & 2 878.1759. **"Kush"**

Inflorescence 3–11 cm. Shorter pedicillated spikelet 25–36 mm: lower glume 22–3mm oblong-lanceolate, rounded on back apex subtruncate-cilliate, 6 ribbed, upper glume longer. 25–3.6 mm. lanceolate, conduplicate, hyaline, margins minutely, acuminate upper lemma 0.6–13 mm, oblong to lanceolate, palea 0.6 × 0.8–14 mm, apex blunt denticulate, pedicel 0.4–0.9 mm, pedicelled spikelet similar, but glume equal; pedicel 1.2–2.5 mm. Anther 22–20 mm. orange.

Specimen cited: NBU campus, Mondal *et. al.* 013. dated 20.04.2020.

Oplismenus burmannii (Retzius) P. Beauvois, Ess. Agrost. 54, 168, 169. 1812: Hook. *f*., Fl. Brit. Ind. 7: 68. 1896; Jansen, Reinwardtia 2.2: 312. 1955; Bor, Grass. Ind. 317. 1960; Noltie, Fl. Bhutan 3(2):686 - 687. 2000. *Panicum burman* Retz, Obs, Bot 3: 10. 1783.

Inflorescence 3–9 cm, axis flexuous, triquetrous, angles ciliate; racemes 5–9, dense the lowest 1–15 cm, axis bearing long cilia Spikelets 2.4–3mm. Lower glume 7–22 × 0.7 mm. lanceolate, narrowly to blunt apex, 3-veined, margins densely ciliate awn 71–12–3 mm. subterminal, minutely antrosely scabrid apex, 5 veined, margins densely ciliate back hairy, awn 34–5 mm Lower floret: lemma 2.4–3 × 0.9–1.1 mm, lanceolate, acuminate, long and short hairy on upperpart of margins 7 veined, awn 0.4–0.9 mm, palea usually absent or linear oblanceolate 21–0.3 mm Upper floret lemma 2.2–2.6 × 0.7 –0.9 mm narrowly lanceolate, acute palea 2–2.4×0.6–0.8 mm; anthers 0.6–1.1 mm.

Specimen cited: NBU campus, Mondal *et. al.* 08 dated 05.04.2019

Oplismenus compositus (L.) P. Beauvois, Ess. Agrost. 54, 168. 169. 1812, Hook. *f*., Fl. Brit. Ind. 7: 66. 1896: Ohwi, Act. Phytotax. Geobot 11 35. 1942, 279, 1948; Jansen, Reinwardtia 22: 311. 1953: Bor, Grass. Ind. 317. 1960; Hara, Fl. E. Himl. 369.1966; Noltie, Fl. Bhutan 3(2):717. 2000. *Panicum compositum* L., Sp. Pl. 1:57. 1753.

Inflorescence 12–23 cm, axis stout, glabrous: racemes 5–10, the lowest 3.7–8 cm, axis triquetrous glabrous or very shortly hairy. Spikelets 3.7–4mm. Lower glume 3.3–3.5 × 1.2–15 mm. lanceolate, tapered upward, sparsely hairy near margins, 5 veined, awn 6–84 mm, upper glume 2.6 –3.2 × 1.3 – 15 mm elliptic acute, hairy near margins or sub glabrous, 7 veined, awn 0.8–5.5 mm 5.5 mm. Lower floret lemma 3.4–3.6 mm, broadly lanceolate, blunty acuminate, hairy on upper part of margins, 9 veined: palea usually absent or linear-lanceolate. 2.7–0.8 mm, anthers 1.2 mm. Upper floret lemma 2.8–3.2 × 0.9–13 mm, oblong-lanceolate, acute, palea 25–2.9 × 0.8–1.1 mm; anthers 1 mm.

Specimen cited: NBU campus, Mondal *et. al.* 09 dated 10.04.2019.

Oryza sativa L., Sp. Pl.ed.1. 333. 1753; Hook. *f*., FI Brit. Ind. 7: 92.1896: Bor, Grass. Ind. 605. 1960; Noltie, Fl. Bhutan 3(2): 517. 2000. **"Dhan"**

Inflorescence 13–17.5 cm, lowest branches single or paired Spikelets 7.52–85 × 3–32. persistent. Sterile lemmas equal, 2–24 × 0.8 mm, triangular, acute, weakly keeled glabrous margins whitish-green.

Fertile lemma 7–8.2 mm, each side 2–22 mm wide, oblong, abruptly acuminate, surface finally reticulately pitted, sparsely hispid on sides above, keel shortly ciliate near apex. Palea 6.7–8 mm, abruptly acuminate each side 12–1.4 mm wide narrowly oblong, keel and sides shortly hispid above anthers 1.8 mm.

Specimen cited: NBU campus, Mondal *et. al.* 04, dated 24.03.2019

Paspalum scrobiculatum L., Mant. Pl. 1: 29. 1767, Hook. *f*., FI. Brit. Ind 7 10. 1896. Hour., Blumea 3: 439 1940; Ohwi, Act Phytotax Geobot. II 40.1942, Bor, Grass. Ind. 340.1960; Noltie, Fl. Bhutan 3(2): 713. 2000.

Spikelets 1.9–2.2 mm pedicels 0.3–0.6 mm. Upper glume 15–18 mm, broadly elliptic, concave. blunt or subacute, glabrous, 5 veined, thing herbaceous, margins unruffled Upper floret lemma broadly elliptic, convex blunt, crustaceous, smooth, margins, inrolled, elapsing palea 1.6–18 × 12–15 mm.

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crustaceous, back flat, margins inflexed, expended in middle; anthers 0.7 mm.

Specimen cited: Gorumara National Park, Mondal *et. al.* 014 dated 21.04.2019

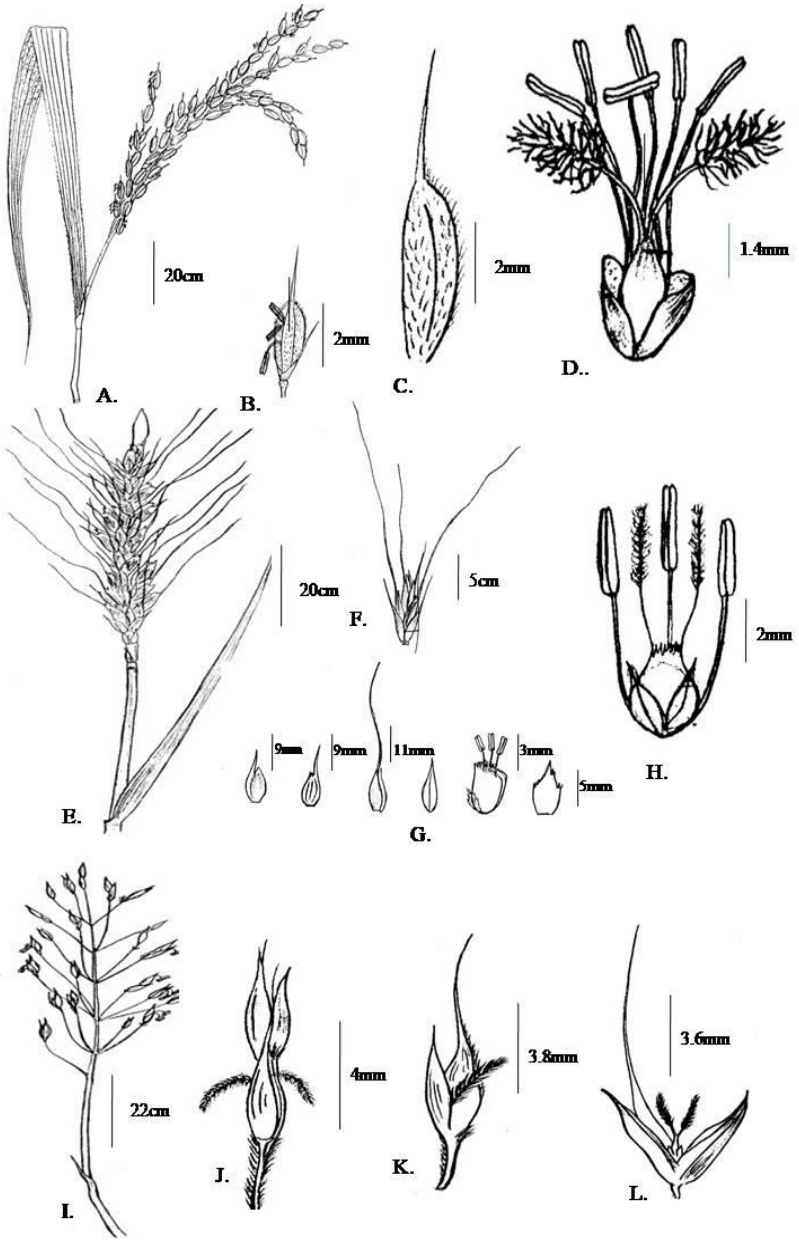

Fig. 2: A-D. *Oryza sativa*, **A.** habit, **B.** spikelet, **C.** lemma, **D.** pistil; **E-H.** *Triticum aestivum*, habit, **F.** spikelet, **G.** glumes, **H.** pistil; **I-L.** *Chrysopogon asiculatus*, **I.** habit, **J.** spikelet, **K & L.** female flower

Saccharum spontaneum Linnaeus, Mant. Pl. 2: 1771; Hook. *f.*, Fl. Brit. Ind. 7: 118. 1896; Obwi, Act Phytotax Geobot. 11 151. 1942; Bor, Grass. Ind. 214. 1960; Noltie, Fl. Bhutan 3(2): 764. 2000. **"Kash"**

Sessile spikelet 3–3.3 mm: lower glume 3–32 mm narrowly to oblong-lanceolate, finely acuminate or apex sometimes rounded. glabrous, 2-veined, lower part golden to reddish-brown, thickened, upper part silver -hyaline, margins ciliate below apex: upper glume 2.7–3.3 mm. lanceolate, acuminate, glabrous, margins fimbriate: lower lemma 26–3mm, lanceolate, margin ciliate, upper lemma reduced, filiform, 1.4–26 mm: palae absent or small, 0.09 mm Pedicelled spikelet similar, pedicel 1.7–2.3 mm anthers 12–2.8 mm.

Specimen cited: NBU campus, Mondal *et. al* 011. dated 14.04.2010.

Triticum aestivum L., Sp. Pl. ed. 1. 85. 1753; Bor, Grass. Ind 679, 1960; Noltie, Fl. Bhutan 3(2): 640. 2000. *Triticum vulgare* Vill., Hist. Pl. Dauph. 2:153.1753; Hook. *f.*, Fl. Brit. Ind. 7: 367. 1897. **"Gaam"**

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Spikelels 12.7 –15 cm excl. awns. Glumes 8.4–9.3 mm, 0.6–2.9 mm. Lemma each half 3–4 mm wide, glabrous or hairy near margins, awn 0.4–62 mm, palea 3.3– 4mm; anthers 2.3–3mm. Rachilla internodes bearing third floret 1.3–2.5 mm.
Specimen cited: Jalpaiguri, Mondal *et. al* 011. dated 14.04.2010.

Conclusion

Present study explored the unique characteristic peculiarities of 14 species of grasses from the terai and duars region. The spikelets of the recorded species are quite fascinating structures and found no lodicule in 3 species like *Digitaria ciliaris*, *Imperata cylindrica* and *Axonopus compressus*. In all cases the stigma is feathery and anther is versatile. The glumes are hairy in *Paspalum scrabicularum*, *Eragrostis unioloides*, *Eleusine indica* and *Imperata cylindrica*. In *Oryza sativa*, the 1st and 2nd glume became suppressed and awn is very acute and hard whereas it is less acute, long and curved in *Triticum aestivum.*

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Review Article

Disease Management in Brassicaceae family through various biocontrol agents: A review

Chitra Kundu, Bijeta Rai, Rewaj Subba and Piyush Mathur*

Microbiology laboratory, Department of Botany, University of North Bengal, Raja Rammohunpur, Dist – Darjeeling, West Bengal, Pin – 734013, India

Abstract

Biological control being an eco-friendly approach against phytopathogens holds a great potential in near future. Severity of chemical-based pesticides have resulted risk to mankind and the environment. The increasing demand for chemical free products all over the world promotes eco-friendly approach such as biological control as a replacement to chemical pesticides. Various bio-formulations of living organisms can be employed to control several plant pathogens. Studies have shown that bacteria, fungi and plants can act as an important source of biocontrol products and have shown positive results in both in-vitro and in-vivo conditions. This review will help us to provide insight towards the potential of various biological entities against major diseases in Brassicaceae along with mechanisms which might be useful in developing various bio-pesticides against plant pathogens for sustainable agriculture.

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Introduction

Brassicaceae (Cruciferae) is one of the largest angiospermic family under the order Brassicales consisting of biennial, annual and perennial plants. Members of this family belongs to various oilseeds, fodder, vegetables and condiment and are good source for vitamins like A, B1, B2, B6, C, E and K (Raza et al. 2020). The genus *Brassica* is known for its agricultural importance and majority of species under this genus provides edible seeds, leaves, roots, stems, flowers and buds (Rakow 2004) along with oil and cattle feed (Ahuja et al. 2011). Species of *Brassica* like *B. napus* and *B. campestris* are grown in many countries like China, Germany, UK and Canada as oil crops (Zhang et al. 2014). *B. napus* ranks third when crop worldwide (Snowdon 2007) comes to important oilseed. A Brassica rapeseed account for about 30% of total oilseed produced and is the second most edible oilseed in India (Aeron et al. 2011).

Phytopathogens are a major problem to agriculture worldwide deteriorating both the quality and quantity of agricultural products. Pathogens are transmitted from one plant to another *via* air, water and living organisms. Various species of *Brassica* are continuously challenged by number of phytopathogens that caused great losses in this crop. Table 1 summarizes the list of major diseases in different Brassicaceae family responsible for crop losses.

To overcome these losses, synthetic chemical pesticides have been extensively utilized for the management of these plant pathogens. However, such pesticides with their continuous application possess serious threat to mankind and to the overall environment. So, in order to tackle such harmful effects of chemical-based pesticides major importance have been given to eco-friendly approach for pest control. Biological control utilises the resources of biological world in order to maintain plant health against pathogens. Various bacteria, fungi and plants are used to ameliorate crop resistance against plant pathogens. A study showed *Bacillus subtilis* LHS11 and FX2 has potential biocontrol ability against *S. sclerotiorum* pathogen that causes *Sclerotinia* stem rot disease in *B.napus* (Sun et al. 2017). In a different study *B.subtilis* and *Gliocladium catenulatum* formulation

*** *Correspondence*** - piyushmathur316@gmail.com

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reduced disease severity *Brassica napus* by 80% against club root caused by *Pieris brassicae* (Peng et al. 2011). Additionally fungal biocontrol agent *Trichoderma* sp. was able to reduce club root in *Brassica chinensis* (Cheah and Page 1997). With this background, the present review attempts to highlight the different classes of biocontrol agents that have been utilized for the management of various diseases in Brassicaceae family.

Different classes of biocontrol agents

Bacteria

A number of endophytic bacteria have been shown to improve plant growth under normal and stress conditions. Such beneficial bacteria demonstrate a positive attribute towards the host plants and impart resistance for the suppression of various bacterial and fungal pathogens. Various strains of *Bacillus amyloliquefaciens* protect economically important plants from different phytopathogens (Daneilsson et al. 2007). The potential of Rhamnolipids (RLs) produced by *Pseudomonas aeruginosa* protects the *Brassica napus* tissues against the ascomycetes *B.cinerea* (Sanchez et al. 2015). Similarly, In *Brassica napus, Bacillus endophyticus* shows an antagonistic activity towards the number of fungal pathogens (Daneilsson et al. 2007). Several biocontrol agents (like *Bacillus pumilus, Bacillus subtilis, Paenibacillus* sp. and several other yeast strains) showed great potential in dealing with *Xanthomonas campestris* pv. *campestris* infections that cause Black rot in many *Brassica* sp. (Assis et al. 1999; Wulff et al. 2002*).* Similarly, *Bacillus pumilus* also shows an antagonistic activity towards *Xanthomonas campestris* in *Brassica oleracea* (Wulff et al. 2002). *Bacillus endophyticus* shows antagonistic activity towards large number of fungal pathogens in *B. napus* (Danielsson et al. 2007). *Microbiospora rosea* suppresses the occurrence of *Plasmodiophora brassicae* in *Brassica rapa* (Lee et al. 2008). Similarly, *Paenibacillus polymyxa* shows antagonism towards *Verticillium longisporum* in *B.napus* (Grane et al. 2003). Similarly, *Bacillus cereus, Bacillus lentimorbus* and *Bacillus pumilus* potential antagonistic activity against black rot disease caused by *Xanthomonas campestris* pv. *campestris* in cabbage plants (Massomo et al. 2004). *Paenibacillus* spp. isolates was able to reduce symptoms of black rot in *Brassica oleracea* var. *capitata* caused by *Xanthomonas campestris* pv. *campestris* (Ghazalibiglar et al. 2016). About 91.1% reduction of lesion-forming petals was observed in *Brassica napus* when pre-treated with *Pseudomonas chlororaphis* PA23 against *Sclerotinia sclerotionum* (Duke et al. 2017). A number of bacteria used as a biocontrol agent to manage different diseases of Brassicaceae family are listed in Table 1.

Fungi

Various strains of fungi have been isolated as biocontrol agents against several plant diseases. Beneficial fungi that include class ascomycetes such as *Trichoderma* sp. which is a common soil inhabitant and basidiomycetes such as *Piriformospora indica* demonstrate a good impact on several pathogens (Kim et al. 2007). *Trichoderma* sp. is able to produce various antibiotics, lytic enzymes such as cellulase, chitinase and hemicellulase which protect the plants against various pathogens. Various species of *Trichoderma* such as *T. asperellum, T. harzianum, T. viride and T. hamatum* acts as strong biocontrol agents against various root and foliar diseases (Nieves et al. 1997). *Trichoderma* sp. can interact with both plant rhizosphere and phyllosphere by several mechanisms which includes antagonism, competition for space and nutrients and release of lytic enzymes which directly inhibits the growth of pathogens (Howell 2003; Harman et al. 2004). *Trichoderma* sp. is found to be very effective in suppressing clubroot of Brassica plants caused by *P. brassicae* (Cheah et al. 1997).

Acremonium alternatum forms an endophytic association with *Brassica* species and facilitates biological activity towards bacterial and fungal pathogens. *A. alternatum* inhibits the development of *Plutella xylostella* and *Brevicoryne brassicae* in *Brassica oleracea*, *B. rapa* and *Arabidopsis thaliana* (Doan et al. 2008; Dugassa-Gobena et al. 1998). *Sclerotinia sclerotiorum B. napus* is highly reduced by *Aspergillus flavipes* (Zhang et al. 2014). *Cladosporium* sp. resists *Spodopteralitura* in *B. oleracea* (Thakur et al. 2013). *Piriformospora indica*, a mycorrhizal like fungi is a useful endophyte (Badge et al. 2010; Baltruschat et al. 2008; Lee et al. 2011) which induces systemic resistance towards *Golovinomyces orontii* in *Arabidopsis thaliana* and *B. rapa* and is regarded as the representative amongst the fungi with spectacular biocontrol potential (Schafer et al. 2007). *P. indica* promotes the plant growth, increased seed productions, stimulates nitrogen accumulation, drought tolerance and induced systemic resistance towards *G. sorontii* in *Brassica*

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rapa and *Arabidopsis thaliana* (Oelmuller et al. 2009; Shahollari et al. 2007; Sirrenberg et al. 2007; Sun et al. 2010). *Fusarium oxysporum* shows antifungal activity towards *S. sclerotiorma* and

Table 1 A list of studies that utilized Bacteria as a biocontrol agent to manage different diseases of Brassicaceae family

Bacteria	Host	Disease	Causal organism	References
Pseudomonas fluorescens	*Brassica campestris*	Stem Blight disease	*Sclerotinia sclerotiorum*	Aeron et al. 2011
Flavobacterium hercynium	*Brassica rapa* subsp. *pekinensis*	Clubroot Disease	*Plasmodiophora brassicae*	Hahm et al. 2012
Bacillus subtilis	*Brassica napus*	Clubroot Disease	*Plasmodiophora brassicae*	Lahlali et al. 2013
Paenibacillus sp.	*Brassica oleracea* var. *capitata*	Black rot disease	*Xanthomonas campestris* pv. *campestris*	Ghazalibiglar et al. 2015
Bacillus amyloliquefaciens subsp. *Plantarum*	*Brassica rapa* subsp. *pekinensis*	Damping off Disease	*Rhizoctonia solani*	Kang et al. 2015
Lactobacillus plantarum strain BY	Chinese cabbage	Bacterial soft rot	*Pectobacterium carotovorum* subsp. *carotoorum*	Tsuda et al. 2016
Zhihengliuella aestuarii	*Brassica juncea* var. *tumida tsen*	Mustard clubroot	*Plasmodiophora brassicae*	Luo et al. 2018
Streptomyces angustmyceticus NR8-2	*Brassica rapa* subsp. *pekinensis*	Leaf spots	*Colletotrichum* sp.;*Curvularia lunata*	Wonglom et al. 2019
Bacillus amyloliquefaciens KC-1	*Chinese cabbage*	Soft rot	*Pectobacterium carotovorum* subsp. *carotoorum*	Cui et al. 2019
Bacillus thuringiensis	*Brassica campestris*	Sclerotiniose disease	*Sclerotinia sclerotiorum*	Wang et al. 2020

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Botryis cinerea in *B. napus* and *Fusarium tricinctum* promotes plant growth in *B. napus* (Zhang et al. 2014). *Metarhizium anisopliae* inhibits the larvae of *Plutella xylostella* in *B. napus* (Batta 2013). Broth culture filtrates of *Aspergillus flavipes* CanS-34A, *Leptosphaeria biglobosa* CanS-51, *Chaetomium globosum* CanS-73 and *Clonostachys rosea* CanS-43 suppressed leaf blight of *B. napus* caused by *S. sclerotiorum* whereas volatile compounds produced by *Fusarium oxysporum* CanR-46 was able to inhibit both *S. sclerotiorum* and *Botrytis cinerea* (Zhang et al. 2014).

Some of the endophytic fungi like *Chaetomium globosum* show in vitro antifungal activity against *S. sclerotiorum* (Chen et al. 2005; Nan et al. 2011). *Leptosphaeria biglobosa* promotes the growth of the plant and also shows the antifungal activity towards *S. sclerotiorum* in *Brassicanapus* (Zhang et al. 2014). *Muscodor albus* shows antagonistic activity towards *Pythium ultimum* in *Brassica oleracea* (Worapong and Strobel 2009). List of fungi used as a biocontrol agent to manage different diseases of Brassicaceae family are given in Table 2.

Table 2 A list of studies that utilized fungi as a biocontrol agent to manage different diseases of Brassicaceae family

Fungi	Host	Disease	Causal organism	References
Acremonium alternatum	*Brassica rapa, Arabidopsis thaliana*	Clubroot disease	*Plasmodiophora brassicae*	Doan et al. 2010
Trichoderma viridae	*Brassica campestris sp. chinensis*	Yellow disease	*Fusarium oxysporum*	Kataoka et al. 2010
Serratia plymuthica HRO-C48	*Brassica napus*	Blackleg disease	*Phoma lingam*	Hammoudi et al. 2012
Trichoderma harzianum	*Brassica rapa, Arabidopsis thaliana*	Root knot	*Meloidogyne incognita*	Ibrahim et al. 2012
Trichoderma harzianum	*Brassica napus*	Powdery mildew disease	*Erysiphe cruciferarum*	Alkooranee et al. 2015
T. harzianum, T. hamatum, T. longibrachiatum	*Brassica napus*	Stem canker disease	*Leptosphaeria maculens*	Dawidziuk et al. 2016

Plant extracts

Plant extracts are very effective and it is used in biological control against several phytopathogens of *Brassica* sp. Acetone extracts from *Cymbopogon citratus* shows effective results in controlling the black rot disease of Brassica. Beside *C. citratus, extracts of Agapanthus caulescens* along with *Paenibacillus sp*. also demonstrate a biocontrol

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activity against *Xanthomonas campestris* pv. *campestris* in *B. napus* (Mandiriza et al. 2018). Extracts of *Chrysanthemam cinerariaefolium* and *Melia azedarach* are used for controlling chinese cabbage disease caused by the diamond black moth (*Plutella xylostella*) and mites like *Phytoseiulu spersimilis* and *Hypoaspis aculeifer* (Kim et al. 2010). Another leaf extract of *Agave americana* has antifungal activity against *Alternaria brassicae*, the causal agent of the disease *Alternaria* blight in Indian mustard *Brassica juncea*. Cabbage aphid (*Brevicoryne brassicae*) and diamond back moth (*Plutella xylostella*) lowers cabbage production and to control these plant extracts of *Ageratum conyzoides*, *Nicotiana tabacum*, *Ricinus communis* and *Casia sophera*, were used (Amoabeng et al. 2013). Extracts of neem (*Azadirachta indica*) along with *Trichoderma harzianum* isolate T-2 was found to be very effective against *Alternaria* blight in radish (Arefin et al. 2019). The small white cabbage butterfly (*Pieris rapae*) and diamond back moth (*P. xylostella*) are the important pests in Brassicaceae, which developed resistance to chemical controls. To manage these pests plant enzyme inhibitors and the proteinaceous compounds extracted from wheat, canola, sesame, bean and Triticale were utilized (Dastranj et al. 2017). Root kno3t nematode disease in *B. rapa* was controlled by *A. indica* and *T. harzianum* (Ibrahim et al. 2012). Extracts of *Moringa oleifera*, *Datura stramonium*, *A. indica* and *Cortonbon plandianum* used was found to be effective against some seed borne fungi (*Aspergillus sp.*, *Rhizopus* sp., *Fusarium* sp., *Alternaria* sp., etc. (Ghosh et al. 2020). Extracts derived from *A. indica* and *Zingiber officinale* were able to activate resistance inducing enzymes in mustard leaves (Ojaghian et al. 2019). List of plants that have been used as a biocontrol agent to manage different diseases of Brassicaceae family are outlined in Table 3.

Table 3 A list of studies that showed positive effects of plant extracts in controlling different diseases of Brassicaceae family

Extract of plant used	Disease	Causal organism	Host	References
Chrysanthemum cinerariaefolium *Melia azedarach*	Chinese cabbage disease	Diamond blackmoth *(Plutella xylostella);* Mite *(Phytoseiulus persimilis)* and *Hypoaspis aculeifera*	Chinese cabbage	Kim et al. 2010
Azadirachta indica	Root knot nematode disease	Root knot nematode (RNT)	*Brassica rapa*	Ibrahim et al. 2012
Ageratum conyzoides; Chromolaena odorata; Synedrellanodiflora	Tri-Trophic insecticidal effect	*Brevicoryne brassicae* *Plutella xylostella*	Cabbage	Amoabeng et al. 2013
Triticum aestivum *Brassica napus* *Sesamum indicum* Bean of triticale		*Pieris rapae* *Plutella xylostella*	Cabbage	Dastranj et al. 2017
Moringa oleifera	Black Rot disease	*Xanthomonas campestris* pv. *campestris*	*Brassica oleracea*	Goss et al. 2017
Cymbopogon citratus; Plutella xylostella	Black rot Disease	*Xanthomonas campestris pv. Campestris*	*Brassica napus*	Mandiriza et al. 2018

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Azadirachta indica; Trichoderma harzianum	Alternaria blight	*Alternaria brassicae*	*Raphanus sativus*	Arefin et al. 2019
Sapindus trifoliatus; Allium cepa	Alternaria leaf spot	*Alternaria brassicae*	*Brassica oleracea* var. *botrytis*	Valvi et al. 2019
Azadirachta indica; Gingiber officinale	Mustard white mold disease	*Sclerotinia sclerotiorum*	*Brassica juncea* var. *tumida*	Ojaghina et al. 2019
Azadirachta indica		*Lipaphiserysimi (kalt)*	*Brassica campestris*	Bhatta et al. 2019
Moringa oleifera; Datura stramonium; Azadirachta indica; Croton bonplandianum		Seed borne microflora	*Brassica* sp.	Ghosh et al. 2020

Conclusions and future perspective

Brassicaceae family is known to harbour large number of economically important plants. Members of this family is considered as a good source for oil, food and feed along with vitamins and minerals. A large number of diseases in this family have led to serious problems resulting in low quality and lesser yield. In order to overcome such losses agriculture is highly dependent on synthetic chemical pesticides. Use of chemical-based products possesses huge risk to mankind and the environment. Therefore, adoption of eco-friendly approach against phytopathogens is necessary. Biological control generally focuses on the use of biological products against plant pathogens which are environment friendly. Various living organisms or their formulation may be utilized to control number of plant diseases as well molecular mechanism behind the resistance induced by these agents should be studies in great detail.

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Review Article

Genetic Resources of Wild Rice (*Oryza rufipogon*) for Biotic and Abiotic Stress Tolerance Traits

Subhas Chandra Roy*

Plant Genetics & Molecular Breeding Laboratory, Department of Botany, University of North Bengal, PO-NBU, Siliguri-734013, WB, India.

Abstract

Rice (*Oryza sativa* L.) is the most important staple food crop of the world; nearly half of the global population depend on it for majority of their dietary intake. Many stresses (biotic and abiotic) have critically affected rice production throughout the world due to global warming, changing climatic conditions and in addition non-durability of biotic resistance gene (s) incorporated into cultivars. Yield increase is not as per the required rate and becomes yield rate is in stagnation. Primary reason of yield stagnation is due to the narrow genetic base in the released varieties. Minimum number of parental lines were utilised to develop new crop varieties which ultimately leads to narrow genetic base. The narrow genetic base in the improved varieties is going to be a main bottleneck for crop improvement program which shield the yield increase. Genetic bottleneck during domestication causes erosion of the genetic diversity in the well adapted cultivars which leads to yield stagnation. Yield plateaus can be surmount through genetic gain by combining the yield related genes/QTLs from different genetic resources of rice germplasm both from local landraces (CLR) and crop wild relatives (CWR). Wild species are the reservoir of genetic diversity with wide adaptability and tolerance to many biotic and abiotic stresses. It is utmost necessary to characterize and conserve rice germplasm for evaluation and effective use of the genetic diversity prevailed in the rice gene pool. Genetic variability in respect to biotic/abiotic resistance is inadequate in the genetic resources of cultivated rice; however, these traits specific genes are available in the unexplored wild species of *Oryza* which are considered as rich source of agronomically important traits including biotic/abiotic traits. Therefore, breeders are trying to identify and transfer of these valuable genes from wild *Oryza* species to improve varieties through pre-breeding method and with the assistance of molecular breeding technology.

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Introduction

Rice (*Oryza sativa* L.) is most important and staple food crop because more than half of the world's population (>3.5 billion) depends on it for their livelihood (Yang and Zhang 2010; Hu et al. 2014; Qian et al. 2016). It is needed to produce double amount of rice by 2050 to feed the more than 9 billion people in this world (Ray et al. 2013; Arbelaez et al. 2015). Total world production was 748 Mt from 163.1 million hectares with productivity of 4.6 tons/hectare (t/ha) in 2016 of which 676.5 million tons was produced by Asian countries. India needs to produce 150 Mt rice by 2030 to feed the increased population with yield increase rate 4.2 t/h (from present rate 3.2 t/h). Production rate in the released varieties has come to the plateaus due to narrow genetic base in the parental lines used in breeding programs (Khush 1997; 2005; Khush et al. 1990; Tanksley and McCouch 1997; Tian et al. 2006). Genetic bottleneck during domestication also causes erosion of the genetic diversity in the well adapted cultivars which leads to yield stagnation (Tanksley and McCouch 1997). Yield plateaus can be surmount through genetic gain by combining the yield related genes/QTLs from different genetic resources of rice germplasm both from local landraces (CLR) and

*** Correspondence** - subhascr2011@gmail.com

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crop wild relatives (CWR). So, it is utmost necessary to characterize and conserve rice germplasm for evaluation and effective use of the genetic diversity prevailed in the rice gene pool. Also needed to reprogram future breeding program to improve and sustain genetic diversity, broadening the genetic base for improvement of agronomically important traits with the help of marker assisted selection (MAS). Breeder could introgress these agronomically important genes/QTLs using knowledge of molecular breeding techniques such as MAS to widen the genetic base for the improvement of yield potentiality as well as quality (Likian and Graner 2012; McCouch et al. 2012; Li and Zhang 2013; Ahmad et al. 2015; Agarwal et al. 2016; Babu et al. 2017; Singh et al. 2018). Genetic variability, heritability and genetic advance and QTLs mapping in rice has been studied in details to analyse the yield and yield components for crop improvement (Kalyan et al. 2017; Tefera et al. 2017; Sandeep et al. 2018; Laxmi and Chaudhari 2019; Roy and Shil 2020). Out of 950 million hectare arable land, 250 million hectare irrigated land is affected by salinity (Shahbaz and Ashraf 2013). Rice is the most sensitive cereal crops to salinity which is highly affected by saline soil conditions and considered as one of the major abiotic stresses (Eynard et al. 2015). More than 163 million hectare lands are used for rice cultivation over 100 countries mainly in South Asia and South-East Asia, because the region is climatically suitable for rice cultivation. Rice is the most important food crop, more than half (½) of the world's population depends on it for their sustainable livelihood. Population growth is increasing day by day and it will reach more than > 9 billion by 2050, and to feed the overpopulation we need to produce nearly double amount of food grains to fulfil the demand (Mammadov et al. 2018). Yield increase is not as per the required rate and becomes yield rate is in stagnation. Primary reason of yield stagnation is due to the narrow genetic base in the released varieties. Minimum number of parental lines were utilised to develop new crop varieties which ultimately leads to narrow genetic base. The narrow genetic base in the improved varieties is going to be a main bottleneck for crop improvement program which shield the yield increase.

Wild Rice as a Reservoir of Agronomically Important Traits

Many stresses (biotic and abiotic) have critically affected rice production throughout the world due to global warming, changing climatic conditions and in addition non-durability of biotic resistance gene (s) incorporated into cultivars (Normile 2008). Previously it was considered that tolerance traits are negatively correlated with yield trait (Strauss et al. 2002; Wise 2007). Although reality is that transfer of diverse resistance traits into cultivars is not always easy task. Sexually compatible donor wild rice species can be used in conventional breeding process otherwise transgenic technique is to be employed to introgress the desired gene (s) from CWR to cultivars. Some poor qualities unwanted traits can be introgressed from the CWR during conventional breeding process due to linkage drag as a result complication arises in the varietal development. There is a hope to use modern biotechnological approaches such as DNA based molecular markers to eliminate the linkage drag. Thus, marker-assisted backcross breeding (MABB) has been employed as competent technique to quickly eliminate the linkage drag with a minimum number of generations (Peng et al. 2014; Vishwakarma et al. 2014). Different kinds of tools and techniques are being used to characterize and dissect the genetic traits prevailed in the wild rice species for introgression into the cultivars such as chromosomal assignment, monosomic alien addition lines, disomic introgression lines, chromosome segment substitution lines (CSSL), and backcross inbred lines (BIL) (Ali et al. 2010; Jena 2010). Wild species of *Oryza* are the genetic resources of many important traits (Yang et al. 2012; Sanchez et al. 2013) such as resistance to biotic and abiotic stresses (salinity, submergence, aluminium toxicity and drought) (Figure 1).

Wide Hybridization to Introgress Wild Gene into Cultivars

Development of CSSL and NIL lines through Pre-Breeding

Huge number of cultivated rice germplasm exist in the world but harbour a limited genetic diversity due to common parents and origin within single species *Oryza sativa* (Zheng et al. 2017). Due to narrow genetic base in these cultivated varieties, they are prone to attack of diseases and pest and less tolerance to abiotic stresses in this climate change scenario which leads to yield loss as a whole. It is needed to broaden the genetic base of the cultivated germplasm to make them sustainable and more efficient in yield potentiality (Zheng et al. 2017). Pre-breeding is one of the prime important approaches to utilize the wild germplasm of rice for

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introgression of novel genes / QTLs / chromosomal segment with important traits to widen the genetic base of the cultivar as well as gaining popularity in rice improvement program. Whole genome sequencing (WGS) research has recognized that a large number of genetic loci have been selected and improved during hybridization and breeding (Huang et al. 2012; Xie et al. 2015; Zheng et al. 2017). Chromosome segment substitution lines (CSSLs) are the genetic construct of wide hybridization in pre-breeding method and can be used as novel genetic stocks to be exploited in breeding program and genomic analysis services to identify and detect the characteristic features of agronomically important traits for crop improvement program (Balakrishan et al. 2018).

Figure 1. Wild rice (*Oryza rufipogon* Griff.) is considered as immediate progenitor ancestor of cultivated rice (*O. sativa* L). Wild rice is growing in natural habitat condition at marshy ditches of Raiganj, Uttar Dinajpur, West Bengal and reservoir of many agronomically important genes/QTLs.

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Linkage Mapping and QTL Analysis

Linkage map will be constructed based on genotypic data of 150 plants of BC2F1 lines using 200 polymorphic SSR markers on all 12 chromosomes. The genetic linkage maps are to be prepared using the Software MapDisto v.1.7.5 (Lorieux 2012; http://mapdisto.free.fr/MapDisto/) and/or IciMapping software v4.1 (www.isbreeding.net). Recombination fraction is transferred to estimate map distance using the Kosambi mapping function (Kosambi 1943; Lorieux 2012). Genotyping by sequencing (GBS, Elshire et al. 2011) is to be conducted in the final generation of CSSL population BC4F2 for fine mapping of the quantitative traits (QTLs).

Improvement of Basmati Rice Varieties Using Marker Assisted Selection

Marker assisted backcross breeding (MABC) has been utilized for the introgression of disease resistance genes such as Xa13 and Xa21 (bacterial blight), genes for Blast, BPH (brown plant hopper) resistance genes and several abiotic stress tolerance components have been introgressed into a number of Basmati rice varieties. Marker assisted backcross breeding (MABC) has been utilized for the introgression of these genes into various rice cultivars Pusa Basmati 1, Pusa Basmati 1121 and Pusa Basmati 6 (Singh et al. 2012; Singh et al. 2013; Singh and Gopalakrishnan 2016). Wild rice *Oryza rufipogon* and *O. nivara* were used for yield enhancement in elite cultivars through introgression line development and QTL mapping (Sudhakar et al., 2012; Swamy et al., 2012). Yield improvement has been achieved through wide crossing by using wild rice *Oryza rufipogon* as a donor parent in elite cultivars (Thalapati et al., 2012; Thalapati et al., 2015). Utilization of "hidden genes" from wild species has emerged as a novel option for enrichment of genetic diversity for productivity traits. Alien gene has been introgressed into popular rice variety Pusa44 for yield enhancement (Gaikwad et al., 2014) from *O. rufipogon*.

Fungal Blast Disease Resistance Rice Varieties

Biotic stress such as blast disease is continued to be the constraint in rice production and becoming a severe problem worldwide in this global warming and climate change scenario (Umakanth et al. 2017). Blast is measured as the most severe and economically crucial disease caused by a fungal pathogen *Magnaporthe oryzae* (*M. oryzae*) (Wang et al. 2014). One of the most identifiable major biotic stresses is the blast disease caused by Magnaporthe oryzae (Umakanth et al. 2017). Although 100 major blast resistance genes (R-genes) have been identified, mapped and their tightly linked DNA markers are available (Miah et al. 2013), only one major gene (Pb1) has been reported for neck blast (Hayashi et al. 2010). In case of blast control, identification of resistance gene (termed as R-gene) is in prime importance, thus many R-genes (more than 100) and about 350 QTLs have been investigated from wild species of rice (Wang et al. 2014; Ashkani et al. 2015; Vasudevan et al. 2015). Some major R gene clusters viz. Piz, Pik, and Pita, were identified and mapped to chromosomes 6, 11, and 12, respectively and some of these are cloned (Wang et al. 2014). Cloned R-genes have been extensively studied at molecular level and have been found to encode nucleotide binding site-leucine-rich repeat (NBS-LRR) proteins (Wang et al. 2014). These types of R genes for blast tolerance have been characterized from wild rice species *O. minuta*, *O. autraliensis*, *O. rufipogon*, and *O. rhizomatis*, and considered as precious germplasm to harbor blast resistance R-genes (Wang et al. 2014). These R genes have been transferred into susceptible varieties and confirmed the effectiveness against rice blast severe attack (Sharma et al. 2012). Durability is not so long if single R-gene has been introgressed, it will break by the various pathotypes within a minimum time span. Therefore, it is suggested to make staking of broad spectrum R-genes to develop more durable resistance varieties with overlapping resistance spectra (Sharma et al. 2014). Therefore, it is necessary to explore new gene pool for R-genes from wild species to get ready before transfer to cultivars. Crop landraces are genetically more dynamic and adaptive equilibrium with both the environment and pathogens (Harlan 1975). Many potential landraces of rice are being replaced by high yielding varieties to meet the food requirements (Umakanth et al. 2017). Despite being less productive they are known to have a high genetic variability for several biotic stresses (Hanamaratti et al. 2008), so they can be explored for rice improvement. Genetic diversity among the rice populations has been precisely assessed by using advance marker technology along with morphological traits (Kumbhar et al. 2015). Molecular markers systems have been successfully exploited by others in rice germplasm

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characterization (Zhang et al. 2011; Liu et al. 2015; Nachimuthu et al. 2015; Anandan et al. 2016). Among the molecular markers SSRs aid in accurately estimating the genetic diversity among rice germplasm and are accounted to be more efficient than single-nucleotide polymorphic markers (SNPs) (Singh et al. 2013; Nachimuthu et al. 2015).

Bacterial blight (BB) resistance rice varieties

Leaf blight is a devastating rice disease caused by bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), yield loss goes as high as 75% in India, Indonesia, and the Philippines (Shakiba and Eizenga 2014). To date near about 41 resistance genes have been identified and out of which eight genes are very extensively characterized for use in breeding program (Ellur et al. 2016). Wild species are the main sources of resistance genes to be used in breeding program. One important bacterial blight resistance gene Xa21, was isolated through positional cloning approach from wild rice species, and found to encode a kinase-like receptor protein (Song et al. 1995). It has been reported that Xa21 is the first gene tagged with DNA markers and used extensively to develop resistant varieties through MAS. More durable resistance gene Xa23, a single dominant gene has been identified from wild rice *O. rufipogon*, with broad spectrum resistance efficacy and considered most promising gene highly resistant to majority of the *Xoo* strains (Zhang et al. 2001; Zhang and Xie 2014).

A new resistance gene, Xa38, has been recognized from *O. nivara* and its exploitation in breeding strategies is expected in near future (Ellur et al. 2016).

Conventional Breeding for the Development of Virus resistance rice varieties

It was reported that approximately 20 different types of viruses can infect rice and a majority of them use insects as vectors for their transmission. Among these two are more important because they cause significant damage to rice production such as rice grassy stunt disease; caused by rice grassy stunt virus (RGSV), and rice tungro disease; caused by two different variant- rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Insect brown planthopper (BPh) is the vector for transmission of RGSV. Infected plant shows stunted growth with a few panicles with deformed grains sometimes no panicle. After screening of rice germplasm both cultivated and wild species, only wild rice *O. nivara* (AA genome, 2n= 24) was identified as a source of virus resistant trait of single dominant gene, Gs. It was the first successful report of transfer of agronomically useful gene (Gs) resistance to RGSV disease from wild rice species to cultivar (Khush et al. 1977). Other wild rice species *O. longistaminata* and *O. rufipogon* reported to be found as a source of resistance gene against tungro-virus diseases and has been used as donor to develop resistant varieties (Khush et al. 2004).

Conclusion

Wild rice germplasm is the good source of agronomically important traits and can be utilized all of these untapped hidden genes associated with many biotic and abiotic stress tolerance traits. Characterization and conservation of these wild *Oryza* species is utmost important for future food security purpose. Climate resilient and disease resistant improved rice varieties may be developed utilizing wild rice species in conventional breeding program.

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Research Article

Nutritional and Antioxidant Properties of the Seeds of *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc. – An Underutilized Legume of West Bengal

***Sabnam Firdousi*[1], *Rakhi Chakraborty*[2] *and Swarnendu Roy*[1,3,*]**

[1] Molecular and Analytical Biochemistry Laboratory, Department of Botany, University of Gour Banga, Malda, West Bengal, India
[2] Department of Botany, A.P.C. Roy Govt. College, Himachal Vihar, Matigara, West Bengal, India
[3] Present Address: Plant Biochemistry Laboratory, Department of Botany, University of North Bengal, Raja Rammohunpur, West Bengal, India (Present Address)

Abstract

The exploration of underutilized crops for nutritional benefits is one of the major strategies to feed the ever-increasing population. There are many nutritionally rich leguminous crops (*Vigna unguiculata*, *Cassia hirsuta*, *Canavalia ensiformis*, *Dolichos biflorus*, etc.) that have remained unexplored for a long time, though could provide a cheap and alternative food source. The present study was conducted to assess the nutritional and antioxidant properties of the seeds of an underutilized legume *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc. The methanolic seed extract showed concentration-dependent radical scavenging activities against DPPH, ABTS, NO, OH and FRAP. The seed extract also showed the presence of nutritional and antioxidative phytochemicals *viz*. alkaloids, saponins, tannins, phenolics, flavonoids, ascorbic acid etc. The presence of a considerably high amount of protein and a low amount of total sugar can also be regarded as beneficial for regular consumption. Moreover, HPLC-MS analysis also revealed the presence of several phenolic derivatives (gallic acid, pyrogallol, chlorogenic acid, catechol etc.) that might also contribute to the antioxidative property of the seeds. Further research on the isolation, purification and characterization of the antioxidative phytocompounds would help decipher their mechanism of action as well as increase the sustainable utilization of the nutritionally rich legume crop.

Keywords: Antioxidants, Legumes, Phenolics, Phytocompounds, Underutilized crops

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Introduction

Legumes are one of the most important crops that have been cultivated worldwide for ages (Messina, 1999). The nutritional value of legumes has long been appreciated and occupied a major part of traditional diets in many countries (Setchell and Radd, 2000). Legumes belong to the family Leguminosae (Fabaceae) which are consumed directly by human beings commonly as mature dry seeds but occasionally as immature seeds enclosed in pods (Adel et al., 1980). The leguminous grains also known as pulses are valued for their high-quality protein with a good amino acid profile (Amarteifio and Mololo, 1998). They also contain complex carbohydrates (oligosaccharides, dietary fibres and resistant starch), vitamins (vitamin-B, folates, ascorbic acid and tocopherols), minerals as well as antioxidants. (Tiwari et al., 2011; Venter and Eyssen, 2001). Legumes are also very good sources of water-soluble vitamins, especially thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), pyridoxine (vitamin B_6) and folate. They are an excellent source of several minerals, including calcium, copper, iron, magnesium, phosphorus, potassium and zinc (Venter and Eyssen, 2001; Mirabelli and Shehab, 2008). In addition to their food value, legumes are important in agricultural systems because of their ability to fix atmospheric

*** *Correspondence* -** swarnendubotany@nbu.ac.in

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nitrogen thereby increasing the overall fertility of the soil (Tiwari et al., 2011).

Recently, population explosion and limitations in crop production pose a big challenge for sustainable utilization of food crops all over the world. Due to scarcity, high cost and unreliable supply of healthy food in developing and underdeveloped countries, the cheap and alternative sources of healthy and nutritious foods need to be explored. The use of underutilized legume plants in this regard can help fight malnutrition associated problems and therefore enhance the overall health status of the rural and economically backward population (Salvi and Katewa, 2016). The use of indigenous and locally cultivated plants has long been an intimate part of many communities worldwide. Also, the use of underutilized species plays a key role in keeping alive the cultural diversity associated with food habits, health practices, religious rituals and social exchanges. Focusing attention on neglected and underutilized species is therefore an effective way to combat micronutrient deficiency, the so-called 'hidden hunger' and other dietary deficiencies, particularly among the economically vulnerable social groups in developing countries (Salvi and Katewa, 2006).

Underutilized plants contribute immensely to food security and serve as means of survival during unfavourable situations of drought, famine, flood etc. (Assefa and Abebe, 2011). They can also supplement nutritional requirements owing to their better nutritional value (Van Andel, 2006). With an alarming increase in human population and depletion of natural resources, it has been felt necessary to explore the possibility of using under-explored plant resources having potential for food, fodder, energy and industrial usage. Many neglected and underutilized species are relatively nutritionally rich and are also adapted to low input agriculture. The erosion of these species can have immediate consequences on the nutritional status and food security of the poor (Vodouh et al., 2012). As the lower income group of the population is particularly vulnerable, it is suggested that attention must be given to easily available, accessible, cheap but nutritious plant protein sources to improve the nutritional status of the low-income group of the population (Iqbal et al., 2006; Khattab et al., 2009). Different types of underutilized legumes have been reported to be used by people including *Vigna unguiculata* ssp. *cylindrica*, *Mallotus subulatus*, *Cassia hirsutta*, *Canavalia ensiformis*, *Cajanus cajan*, *Sphenostylis sterocarpa*, *Vigna subterranean*, *Vigna racemosa*, *Clitoria fairchildiana*, *Cucumis dipsaceus*, *Lupinus angustifolius*, *Afzelia africana*, *Brachystegi acurycoma*, *Detarium microcarpum*, *Mucuna flagellipes*, *Mucuna pruriens*, *Mucuna deeringiana*, *Mucuna monosperma*, *Macrotyloma uniflorum*, *Dolichos biflorus*, *Phaseolus mungo*, *Rhynchosia filipes*, *Vigna trilobata*, *Vigna mungo*, *Atylosis scarabacoides*, *Dolichos trilobus*, *Teramnus labialis*, *Cicer arietinum* (Fernandez and Berry, 1988; Rajyalakshmi and Geervani,1994; Arinathan et al., 2003).

In the present study, an assessment of some nutritional and antioxidant properties of *Vigna unguiculata* seeds – an underutilized legume of West Bengal has been undertaken. The legume though consumed in the villages as a vegetable and rarely as a pulse is not so popular among the people even in smaller towns and cities and is not even considered an important crop from the point of food consumption. Therefore, the analysis of its nutritional and antioxidant properties would reveal some important findings to consider for wider usage as a food crop.

Materials and Methods

Collection of plant material

The seeds of Vigna unguiculata subsp. sesquipedalis (L.) Verdc. (Local name: Borboti) was collected from the market of the Mokdumpur area of Malda District (**Fig. 1a**).

Extraction of seed components for antioxidant analysis

The seeds were washed 2-3 times with distilled water, shade dried for 5-7 days, ground to a fine powder and stored in an airtight container at room temperature in the dark for further use. 10 g of shade-dried powder was extracted with 50 mL methanol in an orbital shaking incubator at room temperature for 48 h (Patil and Gaikward, 2010). The solvent extract was then filtered using Whatman no. 1 filter paper and concentrated at 60 ºC in a hot air oven. After drying, the extract was finally dissolved in dimethyl sulfoxide (DMSO) and stored in a refrigerator at 4 ºC for further use (**Fig. 1b,c**).

Solvent extractive value

The methanolic extractive value of the seed extract was calculated as the percentage yield of the final residue according to the following formula:

Solvent extractive value (%) = W2 – W1 / W2 X 100

Where W1 is the weight of the final residue after extraction (g) and W2 is the weight of powder taken for extraction (g).

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Fig. 1 (a) Seeds of *Vigna unguiculata* subsp. *sesquipedalis*, (b) Powdered seeds, (c) Methanolic extract of seeds

Phytochemical screening of the seed extract

Terpenes
To 2 mL of methanolic extract 2 mL of 5% $FeCl_3$ was added. The formation of yellow-brown precipitate indicated the presence of tannins in the sample (Parekh and Chanda, 2007).

Alkaloids
2 mL of extract was added to 1.5 mL of 1(N) HCl and the mixture was incubated in a water bath. After that, 6 drops of Wagner's reagent were added. The formation of orange precipitate indicated the presence of alkaloids (Ogunyemi, 1979).

Saponins
Aqueous extract of 2 g powder was made and subjected to the frothing test. Frothing persistence indicated the presence of saponins. Later the froth was mixed with a few drops of olive oil. The formation of emulsion further confirmed the presence of saponins (Sofowora, 1993).

Cardiac glycosides
To 2 mL of extract, 1 mL of glacial acetic acid and 1-2 drops of $FeCl_3$ were added followed by 1 mL of concentrated H_2SO_4. The appearance of a brown ring at the interface of two liquids indicated the presence of cardiac glycosides (Trease and Evans, 1989).

Flavonoids
To 2 mL of methanolic extract, a few drops of concentrated HCl was added followed by the addition of 0.5 g of zinc powder. After 3 min, the appearance of magenta-red colour indicated the presence of flavonoids (Parekh and Chanda, 2007).

Phenolics
1 mL of 1% ferric chloride solution was added to 2 mL of extract. The blue colour developed indicated the presence of phenolics (Martinez and Valencia, 2003).

Free amino acids
To 1 mL extract, 0.25% w/v ninhydrin reagent was added and boiled for a few mins. The formation of blue colour indicated the presence of amino acids.

Tannins
2 mL of extract was mixed with 5mL of $CHCl_3$, and 1 mL of conc. H_2SO_4. Reddish-brown colouration at the junction of two layers was observed which indicated the presence of terpenes.

Anthraquinones
5 mL of the methanolic extract was boiled with 5 mL of H_2SO_4 and filtered hot. The filtrate was shaken with 10 mL of $CHCl_3$. The $CHCl_3$ layer was pipetted into another test tube and 1mL of 10% ammonia was added. The resulting solution was observed for the appearance of a rose pink colour which indicated the presence of anthraquinones.

Phlobatannins
To 1mL of extract, 1% aqueous HCl was added and boiled with the help of a hot plate stirrer. The formation of red coloured precipitate confirmed a positive result.

Quantitative evaluation of some nutritional and antioxidative components

Estimation of total and reducing sugar
Total and reducing sugar was extracted following the method of Harborne (1973). 1g of seed powder was extracted in 10 mL of 95% ethanol and the alcoholic fraction was evaporated in a boiling water bath. The residue was re-extracted with ethanol and the process was repeated three times. Then the residue was dissolved in distilled water and the final volume was made up to 5 mL which was then centrifuged at

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5000 rpm for 10 mins. The supernatant was collected and used for estimation.

Estimation of total sugar was done by Anthrone reagent following the method of Plummer (1978). To 1 mL of test solution, 4 mL of Anthrone reagent (0.2% Antrone in conc. H_2SO_4) was added. Then the reaction mixture was cooled under running tap water and absorbance was measured in a colorimeter at 620 nm and the total sugar content was quantified using a standard curve of D-glucose.

Reducing sugar was estimated by the Nelson-Somogyi method as described by Plummer (1978). 1 mL of the test solution was mixed with 1 mL of alkaline copper tartarate solution (2 g $CuSO_4$, 12 g Na_2CO_3 anhydrous, 8 g Na-K tartrate, 90 g Na_2SO_4 anhydrous in 500 mL of distilled water) and heated over a boiling water bath for 20 mins. The reaction mixture was then cooled under running tap water. After that, 1 mL of Nelson Arseno-molybdate reagent was added along with 2 mL of distilled water and mixed vigorously. A blue colour was developed, the absorbance of which was measured in a colorimeter at 515nm. Quantification of reducing sugar content was done using a standard curve of D-glucose.

Estimation of protein

Soluble protein from the seeds was extracted by the previously described standard protocol (Chakraborty et al., 1995). 1g of tissue was homogenized in a pre-chilled mortar and pestle using 5mL of 50 mM sodium phosphate buffer (pH 7.2) and PVP (polyvinyl pyrrolidone) under ice-cold conditions and centrifuged at 10,000 rpm at 4 °C for 15 mins. The supernatant was used for the estimation of protein.

Quantification of soluble protein was done following the method of Lowry (1951). To 1 mL of supernatant, 5 mL of alkaline reagent (A: 2% sodium carbonate in 0.1(N) NaOH and B: 2% sodium potassium tartrate and 1% $CuSO_4$, then mix A and B in 50:1 ratio) was added, mixed well and incubated at room temperature for 15 mins. After incubation, 0.5 mL of Folin-ciocalteau's reagent was added to the mixture and incubated for 10-15 min. The absorbance was measured at 660 nm.

Estimation of total and ortho-dihydroxy phenol

The phenols were extracted by the method given by Mahadevan and Sridhar (1982). 1 g of seed powder was soaked in 5mL of boiling absolute alcohol in dark for 10 mins. After cooling, the sample was crushed with 80% alcohol and then filtered in a dark chamber. The residue was re-extracted with 80% alcohol and then the final volume was made up to 10 mL with 80% alcohol.

Total phenols were estimated following the previously described protocol of Bray and Thorpe (1954). To 1 mL of extract, 1mL of diluted Folin ciocalteu's reagent (1:1 v/v) and 2 ml of 20% Na_2CO_3 solution was added. The reaction mixture was boiled in a water bath for 1 min and then cooled under running tap water and the final volume was made up to 25 mL by adding distilled water. The absorbance was measured at 650 nm in a colorimeter and quantified using a standard curve of catechol.

Estimation of ortho-dihydroxy phenols was done by the method of Arnow (1937). 1 mL of extract was added with 2 mL of 0.5N HCl, 1 mL of Arnow's reagent (10g $NaNO_2$ and Na_2MoO_4 in 100 ml of dH_2O) and 2 mL of 1 N NaOH. A pink colour was developed and the volume of the reaction mixture was made up to 10 mL with distilled water. After vigorous shaking, the absorbance was measured at 515 nm in a colorimeter and quantified using a standard curve of caffeic acid.

Estimation of ascorbate

1 g of seed powder was crushed with 6% trichloroacetic acid and then filtered using Whatman no. 1 filter paper following the protocol of Mukherjee and Choudhuri (1983). The final volume of the filtrate was made up to 10 mL by adding distilled water. 4 mL of extract was added with 2 mL DNPH (2% dinitrophenyl hydrazine). The reaction mixture was kept in a boiling water bath for 10 mins and cooled at room temperature. Then 5 mL of 80% v/v H_2SO_4 was added and absorbance was read at 530 nm. The amount of ascorbate was calculated using a standard curve of ascorbic acid.

Estimation of saponins

Total saponin content was determined based on vanillin-sulphuric acid colorimetric reaction with some modifications (Makkar et al., 2007). About 0.5 mL of plant extract was added with 0.5 mL of distilled water. About 0.5 mL of vanillin reagent (800 mg of vanillin in 10 ml of 100% ethanol) and 2.5 mL of 72% sulphuric acid were added to the mixture and mixed well. This solution was kept in a water bath at 60 ºC for 10 min. After 10 min, it was cooled under ice-cold water and the absorbance was read at 544 nm. The amount of saponins was expressed as diosgenin equivalents (mg DE/ g extract) derived from a standard curve of diosgenin.

Estimation of alkaloids

1g of seed powder was extracted with 40 mL of 10% acetic acid in a 250 mL beaker and covered to stand

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for 4 h. After that, the mixture was filtered and the volume was reduced to one quarter using a water bath. To this sample, concentrated NH_4OH solution was added drop-wise until the precipitation was completed. The solution was allowed to settle and the precipitate was collected by filtration and weighed. The amount of total alkaloid was calculated as the residual weight obtained after extraction (Senguttuvan et al., 2014).

Estimation of tannins

The total tannin content was estimated using the procedure described by Broadhurst et al. (1978). To 0.5 mL of seed extract, 3 mL of vanillin reagent (4% in methanol) was added. Then 1.5 mL of concentrated HCl was added and the mixture was incubated for 15 min. The absorbance was measured at 500 nm (Senguttuvan et al., 2014).

Estimation of total flavonoids

The total flavonoid content was estimated following a previously standardized protocol (Senguttuvan et al. 2014). 1 mL of seed extract was diluted with 0.2 mL of distilled water followed by the addition of 0.15 mL of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 0.15 mL of aluminium chloride (10%) solution was added and allowed to stand for 6 min. Then 2 mL of sodium hydroxide (4%) solution was added and the final volume was made up to 5 mL with distilled water. The mixture was shaken well and allowed to stand for 15 min at room temperature. The absorbance was measured at 510 nm. The appearance of the pink colour showed the presence of flavonoid content. The total flavonoid content was expressed as rutin equivalent mg RE / g extract on a dry weight basis using the standard curve of rutin.

In vitro antioxidant activity of seed extract

DPPH free radical scavenging activity

Different concentrations of plant extracts (0.1mL) were put in the test tube and 2.9 mL of a methanol solution of DPPH (0.1 mM) was added (Blois et al., 1958). The mixture was kept in the dark at room temperature for 30 min and absorbance was measured at 517 nm against a blank. Ascorbic acid was used as standard. The following equation was used to determine the percentage of the radical scavenging activity of each extract.

DPPH scavenging (%) = 100 X (A_0 - A_s) / A_0

Where A_0 is the absorbance of blank and A_s is the absorbance of the sample.

ABTS free radical scavenging activity

The ABTS (2,2-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity was done following the method of Re et al. (1999). The ABTS radical cation ($ABTS^+$) was produced by the reaction of a 7 mM ABTS solution with potassium persulphate (2.45mM). The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. The mixture was stored in the dark at room temperature for 12 h. before use. After the addition of 0.25 mL of extract to 2 mL of diluted $ABTS^+$ solution, absorbance was measured at 734 nm after exactly 6 min. The decrease in absorption was used for calculating scavenging effect values. Ascorbic acid was used as standard. The following equation was used to determine the percentage of the radical scavenging activity of each extract.

ABTS scavening (%) = 100 X (A_0 - A_s) / A_0

Where A_0 is the absorbance of blank and A_s is the absorbance of the sample.

FRAP assay

The FRAP (Ferric reducing antioxidant potential) was determined using a previously described method with slight modifications (Benzie and Strain, 1996). The fresh FRAP reagent was prepared by mixing 500 mL of acetate buffer (300 mM; pH 3.6), 50 mL of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (10 mM), and 50 mL of $FeCl_3 \cdot 6H_2O$ (50 mM). The spectrophotometric measurement was performed at 593 nm and the measurement was monitored with 75 µL of each extract and 2 mL of FRAP reagent.

Hydroxyl radical scavenging activity

The scavenging activity of the extracts on hydroxyl radical was measured according to a previously described method (Yu et al., 2004). To 2 mL of extract, 400 µL of $FeCl_2$ (1 mM), 800 µL of 1,10-phenanthroline (1 mM) and 1 mL of H_2O_2 (0.6 %) were added. The mixture was then homogenized using a vortex and incubated at room temperature for 5 min. The absorbance was read at 560 nm against the blank. The percentage of the radical scavenging activity of each extract was calculated from the equation below:

OH radical scavenging (%) = 100 X (A_0 - A_s) / A_0

Where A_0 is the absorbance of blank and A_s is the absorbance of the sample.

NO scavenging activity

NO (Nitric oxide) scavenging activity was determined according to the Griess-Illosvoy reaction (Garratt, 1964). The reaction mixture contained 2

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mL of sodium nitroprusside (10 mM) in 0.5 mL phosphate buffer (0.5 M; pH 7.4). Various concentrations of the extract (0.5 ml) were added in a final volume of 3 mL. After incubation for 60 min at 37 ºC, Griess reagent [N-(1-Naphthyl) ethylenediamine (0.1%) and sulphanilic acid (1%) in H_3PO4 (5%)] were added. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with N-(1-Naphthyl) ethylenediamine was measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. The scavenging ability (%) of the nitric oxide was calculated using the formula:

NO scavenging effect (%) = 100 X (A_0 - A_s) / A_0

Where A_0 is the absorbance of blank and A_s is the absorbance of the sample.

HPLC of phenolics

Extraction of total phenolics from the dried samples for HPLC analysis was done following the standard protocol with slight modifications (Pari and Latha, 2005). 2 g of powdered sample was soaked in 10 mL of methanol and kept overnight in dark. After 12 h of soaking, the suspension was filtered and the filtrate was completely evaporated using a rotary evaporator at 40°C and lyophilized. The lyophilized extract was re-dissolved in 1 mL of HPLC grade methanol and filtered through a millipore membrane filter (0.45µm). HPLC analysis of total phenolics present in the sample was done following the method described by Pari et al. (2007). The analysis was done using High-Performance Liquid Chromatography (Shimadzu) equipped with HPLC pumps (model LC 10ATVP), UV-Vis detector (SPD-10AVP) and C18 column. The flow rate of 1mL/min, injection volume of 20 µL and binary gradient elution of HPLC grade acetonitrile-water–acetic acid (5:93:2, v/v/v) [solvent A] and acetonitrile-water–acetic acid (40:58:2, v/v/v) [solvent B], starting with solvent B from 0 to 100% over 20 min were applied. The separation of compounds was monitored at 280 nm. The identification of the phenolic compounds was done using the correlations with the standards such as gallic acid, pyrogallol, resorcinol, catechol, catechin, chlorogenic acid, caffeic acid and vanillic acid. The retention time of the standard phenolics are given below:

Table 1 Retention time of standard phenolic compounds

Sl. No.	Phenolic standards	Retention time (in mins)
1	Gallic acid	5.33 - 5.34
2	Pyrogallol	5.75 - 5.77
3	Resorcinol	9.06 - 9.07
4	Catechol	10.15 - 10.25
5	Catechin	14.91 - 14.95
6	Chlorogenic acid	15.65 - 15.69
7	Caffeic acid	16.64 - 16.68
8	Vanillic acid	17.23 - 17.54

Results

Qualitative screening of the seed extract

The phytochemical screening of the seed extract revealed the presence of several phytoconstituents *viz.* tannins, alkaloids, saponins, cardiac glycosides, flavonoids, phenolics, phlobatannins, amino acids, and protein was observed. Among the phytoconstituents, a stronger presence of phenols, flavonoids, alkaloids, tannins, terpenes, anthraquinones and proteins as enlisted in Table 2.

Antioxidant and nutritional status of seed extract

Methanolic extractive value of Vigna seed powder was calculated to be 9% which means that approximately 9 g of phytoconstituents could be extracted using methanol from 100 g of the crude powder. Among the secondary metabolites, a considerable amount of phenols, flavonoids and alkaloids was observed (Table 3). The amount of phenols (1.7 ± 0.11 g/100g) was the highest among

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the secondary metabolites followed by that of the alkaloids (1.14 ± 0.06 g/100g). Other antioxidants like ascorbic acid (0.43 ± 0.09 g/100g) were also present in a significant amount. Quantitative estimation showed that the amount of proteins (8.15 ± 0.5 g/100g) in the seed extract was highest in comparison to the other nutritional components (Table 3). Other nutritional components like sugars, fats and free fatty acids were also quantified and presented in Table 3.

Table 2 Phytochemical screening of the seed extracts

Phytoconstituents	Qualitative tests	Observation	Inference
Tannin	Ferric chloride test	Yellow-brown precipitate	++
Alkaloids	Wagners test	Orange precipitate	++
Saponins	Frothing test	Formation of frothing bubbles	+
Cardiac glycosides	Keller-Kiliani test	Violet ring bellow the brown ring	+
Flavonoids	Shinoda's test	Pink colour	++
Phenolics	Folin-ciocalteau test	Blue colour	+++
Phlobatannins	HCl test	Red precipitate	+
Amino acids	Ninhydrin test	Blue colour	+
Terpenes	H_2SO_4 test	Reddish-brown precipitate	+
Anthraquinolins	Ammonia	Rose pink	+
Protein	Biuret test	Violet colour	++

+++ Strong presence; ++ Moderate presence; + Low presence

In vitro antioxidative activity of the seed extract

DPPH radical scavenging activity

The DPPH scavenging activity of the seed extract showed a concentration-dependent activity as shown in **Fig. 2a**. The standard antioxidant ascorbic acid showed a relatively higher DPPH radical scavenging activity. The DPPH scavenging of *Vigna* seed extract ranged from 0-40% for the tested concentrations; whereas the scavenging activity of ascorbic acid ranged from 90-100%. Here IC_{50} value could not be calculated. The inhibition percentage against the concentration of each extract required to reduce 25% of the DPPH radical was therefore determined. The IC_{25} value for *Vigna* seed extract was calculated to be 3.9 mg/mL.

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ABTS cation scavenging activity

The ABTS scavenging activity of the seed extract was comparatively better in comparison to that of the DPPH scavenging activity. The ABTS scavenging activity of ascorbic acid was relatively higher than the seed extract (**Fig. 2b**). The ABTS scavenging of *Vigna* seed extract ranged from 10-80% for the tested concentrations, whereas the scavenging activity of ascorbic acid ranged from 80-100%. The IC_{50} value for the *Vigna* extract was calculated to be 0.48 mg/mL.

Table 3 Amount of some important antioxidant and nutritional components present in seed extract

Parameters quantified	Amount (g/100g)
Antioxidants components	
Total phenol	1.7 ± 0.11
Ortho-dihydroxy phenol	0.31 ± 0.04
Flavonoids	0.15 ± 0.05
Saponins	0.011 ± 0.006
Tannins	0.015 ± 0.007
Ascorbic acid	0.43 ± 0.09
Alkaloids	1.14 ± 0.06
Nutritional components	
Protein	8.15 ± 0.5
Total sugar	1.2 ± 0.07
Reducing sugar	0.081 ± 0.05
Fats	0.5 ± 0.03
Free fatty acids	0.08 ± 0.007

NO radical scavenging activity

The NO radical scavenging activity of the seed extract compared to ascorbic acid is shown in **Fig. 2c**. The NO radical scavenging activity of *Vigna* seed extract ranged from 5-30% for the tested concentrations, whereas the scavenging activity of ascorbic acid ranged from 78-92%. Here IC_{50} value could not be calculated. The IC_{25} value for the extract was determined to be 0.53 mg/mL.

OH radical scavenging activity

The OH radical scavenging activity of the extract compared to ascorbic acid is shown in **Fig. 2d**. The OH radical scavenging activity of *Vigna* seed extract ranged from 42-80% for the tested concentration, whereas the scavenging activity of ascorbic acid ranged from 4-62%. Therefore, the OH radical scavenging activity was somewhat comparable to ascorbic acid. The IC_{50} value for the seed extract was calculated to be about 0.48 mg/mL.

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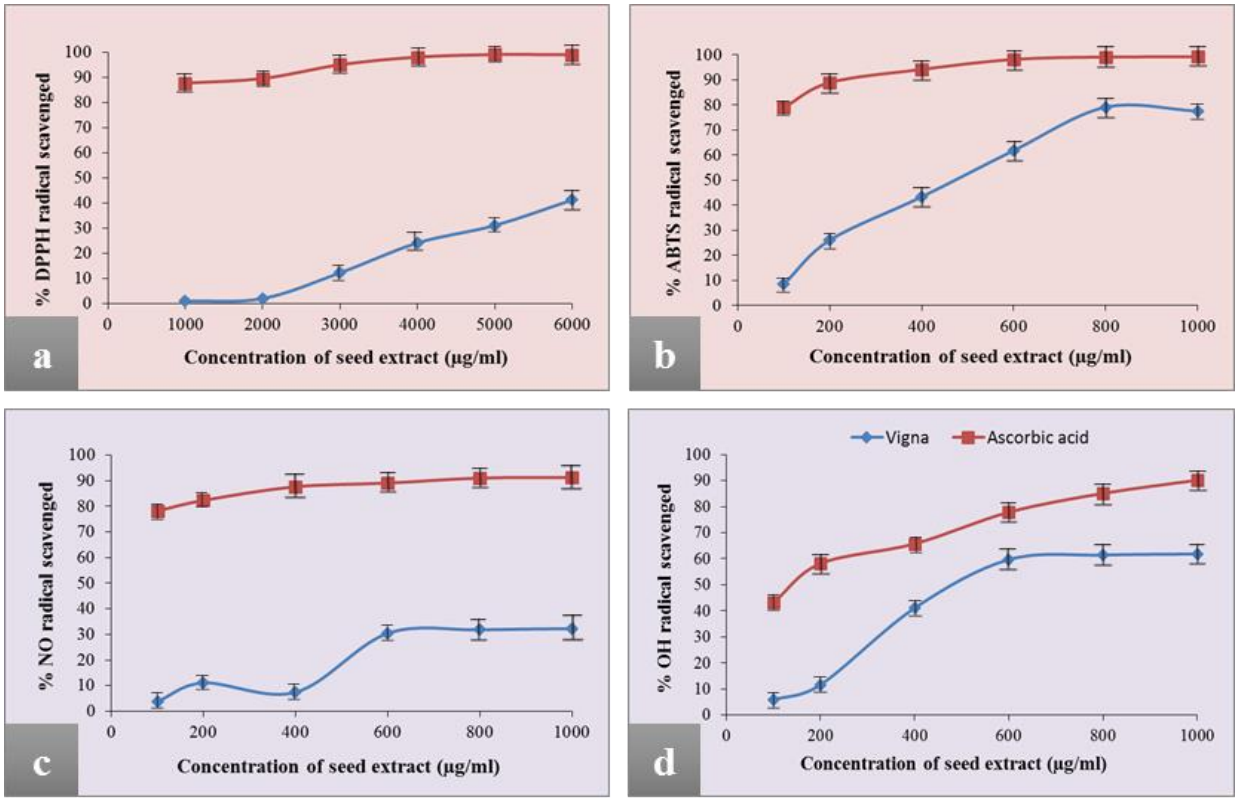

Fig. 2 (a) DPPH scavenging activity, (b) ABTS scavenging activity, (c) NO radical scavenging activity (d) OH radical scavenging activity of the *Vigna* seed extract.

FRAP activity

The scavenging effect of the FRAP radical assay also showed a concentration-dependent activity which was calculated using a standard curve of $FeSO_4$. The FRAP activities of ascorbic acid and *Vigna* seed extract were determined at a concentration of 1mg/mL. Ascorbic acid showed relatively higher FRAP activity (Table 4). The reducing power of seed extract was however found to be 0.053 Fe^{+2} equiv./mg extract.

Table 4 FRAP activities as vcalculated using standard curve of $FeSO_4$

Samples	Conc. of sample (mg/mL)	O.D. (1mg/mL)	Conc. from SC (mM Fe^{2+} equiv.)	FRAP (mM Fe^{2+} equiv./µg extract)
Vigna	1	1.364	1.074015748	0.053700787
Ascorbic acid	1	1.924	1.51496063	0.075748031

HPLC analysis of phenolics

The HPLC chromatogram of the *Vigna* seed extract has shown the presence of some phenolic compounds (**Fig. 3**). Among these some of these compunds has been identified as gallic acid, pyrogallol, catechol, chlorogenic acid, and caffeic acid (peak nos. 5,6,18 31, 34) based on the retention time of standard compounds as shown in Table 5.

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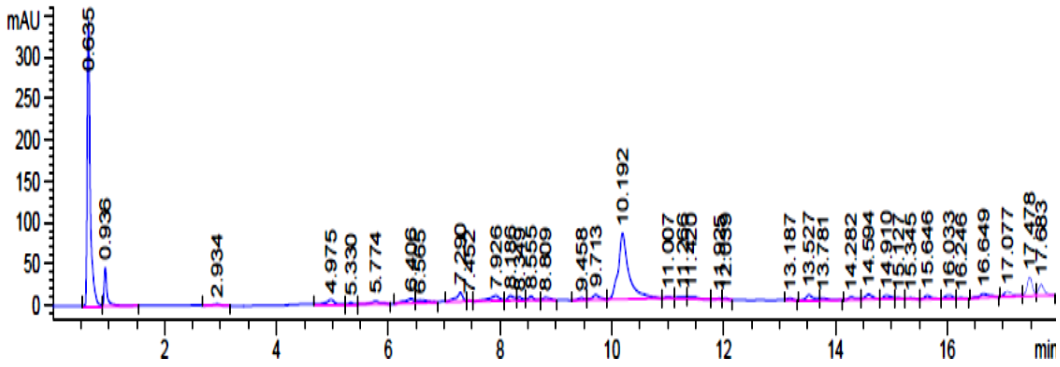

Fig. 3 HPLC profile of phenolics extracted from *Vigna* seed extract.

Table 5 Details of major peaks detected with retention time and peak area

Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Area %
1	0.635	0.0566	1316.45032	33.5963
2	0.936	0.0563	176.71410	4.5098
3	2.934	0.1738	17.21987	0.4395
4	4.975	0.1257	47.35107	1.2084
5	5.330	0.0829	6.31764	0.1612
6	5.774	0.1459	38.93552	0.9936
7	6.406	0.1500	61.49410	1.5694
8	6.565	0.1786	40.61675	1.0366
9	7.290	0.1218	95.85567	2.4463
10	7.452	0.0635	5.38934	0.1375
11	7.926	0.1664	82.05089	2.0940
12	8.186	0.1173	44.81142	1.1436
13	8.349	0.0805	21.68813	0.5535
14	8.555	0.0937	26.04545	0.6647
15	8.809	0.1185	26.35175	0.6725
16	9.458	0.0997	20.73409	0.5291
17	9.713	0.1332	57.09259	1.4570
18	10.192	0.1804	1039.02869	26.5164

Discussion

Legumes are known to be a good source of plant proteins. According to Singh (2017) pulses are an excellent source of protein, sugars, dietary fibre, vitamins and minerals. In the present study, the crude protein content of *Vigna unguiculata* subsp. *sesquipedalis* – an unconventional legume was found to be the highest among all the nutritional and antioxidant components, which is a characteristic feature of the legume seeds. This has been realized that the protein content of the underutilized legume is close to that of other legumes as reported by Bravo et al. (1999) for other lesser-known pulses in India. Therefore, the seemingly higher content of proteins in *Vigna* seeds suggests the potential of these lesser-known legumes being used as alternative sources of protein for the seemingly underprivileged population. Also, the total sugar and reducing sugar content of the *Vigna* seeds were found to be considerably lower enhancing the overall nutritional aspect of the legume.

According to Zia-Ul-Haq et al. (2013), legumes like cowpea are rich in polyphenolic compounds more than other leguminous seeds. In the present study, the presence of a significant number of polyphenolic compounds like phenols, flavonoids and tannins in the *Vigna* seed extract was found. It has also been known that polyphenolic compounds also have beneficial effects due to their antioxidant activity and the ability of phenolic substances including phenolic acids to act as antioxidants has been extensively investigated (Rice-Evans et al., 1997). Tannin is also known to exert beneficial nutritive effects by its ability to form a complex with

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dietary proteins and inhibit endogenous proteins, such as digestive enzymes (Liener, 1994). Researchers have even demonstrated that intake of food rich in flavonoids protects humans against diseases linked with oxidative stress (Shahidi et al., 1995).

Phenolic compounds also consist of phenolic acids produced in plants, and the abundance of these chemicals depends on species and stage of growth (López-Amorós et al., 2006). The presence of phenolics also confers the major proportion of the antioxidant potential of the plant extracts. Sebei et al. (2013) determined eight phenolic compounds in peanut kernels including caffeic acid, dihydroxyphenylacetic acid, syringic acid, p-coumaric acid, rutin trihydrate, naphtoresorcinol, trans-2-hydroxycinnamic acid and dihydrate quercetin. In addition, p-hydroxybenzoic acid and chlorogenic acid were also detected in both peanut kernels and skin (Win et al., 2011). In the present study, the HPLC analysis of phenolics has confirmed the presence of several phenolic compounds in the *Vigna* seed extract.

Apart from being a source of plant proteins, legumes have been known to be rich in polyphenolics making them a very good candidate for free radical scavenging. The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical is a stable free radical, which has been widely accepted as a tool for estimating the free radical-scavenging activities of antioxidants (Park et al., 2005). Similarly, the ABTS assay is very useful for the assessment of total antioxidant capacity. It is based on the inhibition by antioxidants of the absorbance of the radical cation 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate), which has an absorption at 734 nm (Igbinosa et al., 2011). Ferric reducing/antioxidant power (FRAP) reflects total antioxidant power involving the single electron transfer reaction (Igbinosa et al., 2011). Nitric oxide (NO) is a key signaling molecule that plays a crucial role in the pathogenesis of various diseases associated with inflammation (Gates et al., 2008). The OH radical generated by the Fenton reaction is a highly reactive free radical. This free radical can be formed from hydrogen peroxide and the superoxide anion and may be generated in the human body under certain physiological conditions (Lee et al, 2004). In the present study, the determination of free radical scavenging activity of the seed extract pointed towards a considerable degree of antioxidant properties present in the seeds. Among the free radicals, the seed extract showed a comparatively better result in the scavenging of ABTS and NO radicals. However, the results were not comparable to the standard antioxidant - ascorbic acid, this may be due to the use of the standard antioxidant in its purest form whereas the antioxidant components in the seed extracts were present along with the other components. Therefore, the purification and identification of antioxidant components from the seed extract will be important to understanding the free radical scavenging attributes of the seed extract.

Conclusion

From the above experimental results, it is evident that the seed extract of *Vigna unguiculata* subsp. *sesquipedalis* showed considerable antioxidant activities *in vitro*. The seed extract showed concentration-dependent scavenging of free radicals viz. DPPH, ABTS, NO, OH and FRAP. The presence of active phytochemicals like alkaloids, phenolics, flavonoids, saponins, tannins etc. further confirm the nutritional benefits of the seeds. HPLC-MS analysis of phenolics also showed the abundance of several phenolic derivatives *viz.* catechol, pyrogallol, gallic acid, chlorogenic acid etc. which might be responsible for the antioxidative properties. The seeds were also found to be considerably rich in protein, whereas the amount of total sugar and reducing sugar was found to be comparatively lower. Therefore, the results suggest that the underutilized legume *Vigna unguiculata* subsp. *sesquipedalis* can be used as a nutritionally rich alternative food in our

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daily diet. Further research on the isolation, purification and characterization of the antioxidative active compounds would help upgrade the nutritional profile of this underutilized legume.

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Review Article

Zinc Oxide Nanoparticles: Different synthesis approaches and applications

Sudipta Kundu, Md. Salman Haydar and Palash Mandal*

Nanobiology and Phytotherapy Laboratory, Department of Botany, University of North Bengal, Siliguri, West Bengal, 734013, India

Abstract

Nanotechnology deals with the synthesis and usage of materials with nanoscale dimension (1-100 nm). Nanoscale dimensions of the particles provide large surface to volume ratio and thus very specific properties. Synthesis of zinc oxide nanoparticles (ZnO NPs) has gained prime importance in recent arena due to its high excitation binding energy and large bandwidth and it has potential applications like anti-diabetic, antibacterial, anti-inflammatory, antifungal, wound healing, antioxidant and optical properties. Zinc (Zn) is a common mineral element in nature which plays an immense role in many biological processes. It is defined as an essential trace element or micronutrient which is very much crucial for the normal growth and the development of all higher plants as well as animals. Zinc directly involves in enzyme function associated with the photosynthesis and energy process in plants. It also plays an important role in maintenance of membrane integrity, formation and production of growth hormone, insulin, thyroid etc. Due to the involvement of large rate of toxic chemicals and requirement of extreme environment, chemical and physical methods of nanoparticle synthesis often became inappropriate. Whereas, green methods are used in a wide range of biological samples including plants, fungus, bacteria, and algae, which act as both reducing and capping agent. Biologically synthesized zinc nanoparticles have been reported for versatile applications in the field of medicine and pharmacy, for bio-imaging and bio-sensor production, in gene therapy and drug delivery system. Zinc nanoparticles also play vital role in agricultural sector including plant growth and development, enhancement of crop yield and post-harvest processing. In spite of being great potential of ZnO NPs for abiotic and biotic stress management, research works in this field is considerably less. This review described the summary of the recent works in the synthesis mechanism, characterization techniques, and applications of biosynthesized ZnO NPs in medicine and agriculture with special reference to application on plant growth, development and abiotic stress management.

Keywords: Zinc oxide nanoparticles, Nanoparticles, Green synthesis, Plant growth, Abiotic stress

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Introduction

In modern science, one of the rapidly developing concepts in last decade is nanotechnology (Kalpana et al. 2018). The nanomaterials embrace very distinct physiochemical properties, which have the potential to develop new age technology, systems, devices, structural forms, and engineering platforms which leads to the wide-ranging variety of disciplines (Mirzaei et al. 2017; Arruda et al. 2015). Nanomaterials are the specialized nanoscale particles of size below 100 nm with improved conductivity, nonlinear optical performance, actively catalytic and have chemical stability due to their large surface to volume ratio (Agarwal et al. 2017). Nanoparticles can be synthesized through conventional methods (physical synthesis and chemical synthesis) in efficient way but they often require stabilizing agents which may lead to toxicity in the nature and environment. To deal with the problems, incorporation of biological or green way of nanoparticles (NPs) synthesis arises which serves as an alternative environment friendly, non-toxic, economically beneficial approach. Extracts of

* ***Correspondence*** - pmandalbotppprl@nbu.ac.in

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biological elements especially plants act as both reducing and capping agent during reduction of metallic precursor to nano-form as a result of which involvement of eternal capping agent is not required in this process (Salam et al. 2014). Nanoparticles including metallic and metal oxide shows beneficial effect on health (Bhattacharya et al. 2008) having a wide range of applications such as for medical imaging (Nune et al. 2009), drug delivery (Nasimi et al. 2013), cancer therapy (Siddique and chow 2021), textile (Yetisen et al. 2016), renewable energy (Hussein 2015), environment (Martínez et al. 2021), electronics (Matsui 2005), food, agriculture (Paramo et al. 2020) etc.

Metallic nanoparticles (such as Ag, Au, Pt, Cd etc.) and metal oxide nanoparticles (such as ZnO, TiO_2, ZrO_2, CeO_2, etc.) are the general classification of nanoparticles. Among different metal oxide nanoparticles, ZnO attracts great attention due to their special physiochemical properties, along with unique shape and size (Theerthagiri et al. 2019). According to ancient literature, ZnO had been used since at least two millennia B.C. in ancient Egyptian civilization and later in Rome as ointments for the treatment against skin diseases and wounds (Frederickson et al. 2005). Currently ZnO is being used across different industries such as brass production (Habashi 2001; Biswas 1987), rubber industry (Eastaugh et al. 2008), ceramic industry (Moezzi et al. 2012), concrete manufacturing (Brown 1957), electronic devises, food and others (Borysiewicz 2019).

Several researches described ZnO as a structurally functional, strategic, promising and resourceful material with broad range of applications (Neumark and Kuskovsky 2007). ZnO NPs typically contain neutral hydroxyl groups attached to their surface, which play important role in surface attachment and structural change behaviour (Qu and Morais 1999; 2001). The isoelectric point of ZnO NPs ranges in between 9-10, which indicate ZnO NPs contain a strong positive surface charge under physiological condition. ZnO NPs have great advantages in catalytic reaction due to their large surface to volume ratio (Huang et al. 2006). ZnO is a one-dimensional nanoparticle which exhibits interesting electronic and optical properties and due to their low dimensionality, they show quantum confinement effect (Baruah and Dutta 2009). One of the interesting facts is that the cancer cells frequently contain high concentrations of negatively charged phospholipids on their outer membrane with high membrane potentiality; therefore, interaction with positively charged ZnO NPs is probably achieved by intermingling with electrostatic interactions, thus promoting cellular nutrition uptake, phagocytosis and ultimate cytotoxicity (Abercrombie and Ambrose 1962; Bockris and Habib 1982; Papo et al. 2003). ZnO NPs exhibits piezoelectric property, which is very much advantageous for the fabrication of devices, such as electromagnetic coupled sensors and actuators (Minne et al. 1995).

Zinc serves as essential micronutrients in plant system (Marschner 1993). Zn act as cofactor of more than 300 proteins, among of which the majority are zinc finger proteins, DNA and RNA polymerases (Coleman 1998; Lopez Millan et al. 2005). Also, it is the only metal which is present in all the six class of enzyme *viz.* oxidoreductase, transferase, hydrolases, lyases, isomerases and ligases (Gupta et al. 2016). Zn being a part of the structural and catalytic unit regulates the activity, function, stabilization and folding of the various proteins and enzymes. It also involves in hormone regulation (in tryptophan synthesis Zn is very essential component which is a precursor of IAA) (Castillo-González et al. 2018), mitogen-activated protein kinase regulation which directly involves in signal transduction pathway (Lin et al. 2005; Hansch and Mendel 2009), control PS II complex in photo-inhibition process (Monnet et al. 2001; Bailey et al. 2002; Lu et al. 2011), and are found to involve in the Rubisco activity (Peck and McDonald 2010). Zinc has impressive role in maintenance and regulation of gene expression corresponding to environmental stress (such as high temperatures, high light intensity) tolerance in plants (Cakmak 2000).

Globally 49% of the soils are deficient of zinc making it the most noticeable micronutrient deficiency in the World (Graham 2008). Following the trend, on an average, 36.5% of the Indian soils are potentially deficient of zinc being highest than the rest of the micronutrients (Shukla et al. 2018). For restoration of this deficiency, several commercial products are used which are mainly synthetic chemical fertilizers which leads to soil mineral imbalance, abolishing soil texture and fertility and showing long-term negative effect in our ecosystem (Elemike et al. 2019). To overcome this problem, supplementation of biofertilizers might play important role in the improvement of crop production and soil fertility (Bhardwaj et al. 2014), but large-scale industrial production of biofertilizers and their appropriate usage are not easy. To deal with it, nanotechnology generated nano-fertilizer arises which serves as the latest technology in precision agriculture. Specifically, use of zinc oxide nanoparticles can able to restore the zinc deficiency

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in soil and also can able to enhance plant growth and development (Prasad et al. 2012; Laware et al. 2014; Singh et al. 2016; Elizabath et al. 2017). Also zinc oxide nanoparticles could be used in management of several abiotic stresses viz. drought stress (Taran et al. 2017; Hassan et al. 2020; Sun et al. 2020) and salt stress (Sanaeiostovar et al. 2012; Soliman et al. 2015; Alharby et al. 2016). This present review describes the synthesis of zinc oxide nanoparticles, its characterization, its application in plant growth and development and abiotic stress management with detailed description of uptake, translocation and accumulation of zinc in the plant system.

Synthesis methods of zinc oxide nanoparticles

There are mainly three strategies for synthesis of nanoparticles *viz.* (a) Physical synthesis, (b) Chemical synthesis and (c) Biological synthesis (Dhand et al. 2015) (Figure 2). Among these Physical and Chemical way of NPs synthesis is most conventional one but it possesses certain level of toxic substances (Li et al. 2011). On the other hand, biological or green way of synthesis is quite advanced with special properties such as cost effectiveness, eco-friendly, easily available and less-toxic in nature (Ingale et al. 2013).

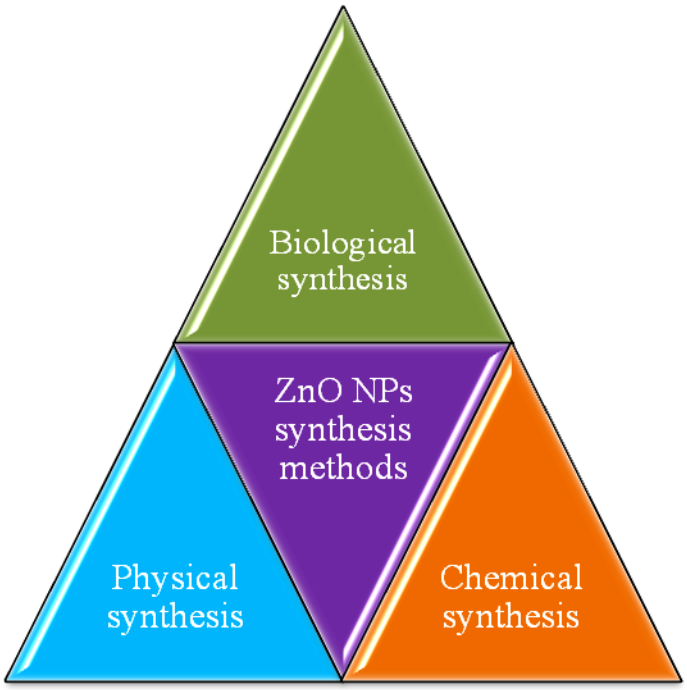

Fig. 1 Different strategies of zinc oxide nanoparticles synthesis

Physical method of zinc oxide nanoparticles synthesis

In this process, the physical forces are used as an external agent for the formation of stable and distinctly shaped nanoparticles, e.g., colloidal dispersion, physical fragmentation, vapour condensation, etc. (Krupa 2016). In case of ZnO NPs synthesis, plasma method, laser ablation method and thermal evaporation are the most commonly used physical strategies (Brintha and Ajitha 2015). Laser ablation method offers some unique benefits because it allows properly sized, shaped and well-purified ZnO nanoparticles (Ma et al. 2013), where the ablation time and wavelength are important factor for ZnO NPs synthesis (Manzoor et al. 2015). In thermal evaporation method powder material are vaporized at very high temperature and the vapour phase condensed under high pressure to obtain nanoparticles (Happy et al. 2017). ZnO nanoparticles synthesized through physical method shows a strong potential photo-catalytic activity which is very much beneficial for photo-degradation (Parita Basnet et al. 2018). Physical methods of nanoparticles synthesis have some limitations because of the large expensive equipments with high pressure and temperature, also this process often leads to irregular particle formation (Chandrasekaran et al. 2016).

Chemical methods of zinc oxide nanoparticles synthesis

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In chemical method of nanoparticles synthesis, one or more chemical reactions are carried out through which the final product of nanoparticles synthesized. Chemical synthesis methods involve two major phases i.e., gas phase and liquid phase. The gas phase is divided into pyrolysis and gas condensation method and liquid phase is sub-divided into precipitation method, hydrothermal method, colloidal method, sol-gel method and oil emulsion method. (Naveed UlHaq et al. 2017). During ZnO NPs certain stabilizers (citrates or polyvinyl pyrrolidone) are used to control the morphology and avoid agglomeration of ZnO NPs (Naveed et al. 2017). However, the chemical method of synthesis has some sort of drawbacks because it requires high energy, toxic chemicals, highly expensive equipments and several researches showed that there are certain traces of toxic reagents in the chemically synthesized zinc nanoparticles which could be hazardous for application in several sectors (Anshuman et al. 2014).

Biological method of zinc oxide nanoparticles synthesis

Biological synthesis or green synthesis approaches provide an environment friendly, low-toxic, cost effective and efficient protocol to synthesize and fabricate the NPs. These methods employed several biological elements *viz.* bacteria (Klaus et al. 1999; Rohet al. 2001; Nair et al. 2002; Yong et al. 2002; Husseiny et al. 2007), fungi (Mukherjee et al. 2001a: Mukherjee et al. 2001b; Ahmad et al. 2005), yeast (Kowshik et al. 2003), actinomycetes (Ahmad et a., 2003a; Ahmad et al. 2003b; Sastry et al. 2003), virus (Shenton et al. 1999; Lee et al. 2002; Merzlyak et al. 2006) and plant extracts for the production of ZnO nanoparticles. Despite of the advantages of using microorganisms as reducing agent in synthesis of ZnO NPs, it also may contain some number of toxic substances which directly or indirectly affect the quality of ZnO NPs (Guldiken et al. 2018). Whereas, plants contain several types of phytochemicals or secondary metabolites such as methyl xanthenes, phenolic acids, tannins, alkaloids, flavonoids etc. have been reported as very good reducing agents of metallic precursors (Altemimi et al. 2017). The use of plant sample has some benefits over the others such as it is safe, cost-effective, eco-friendly, non-hazardous, easy to handle and synthesis (Abdul et al. 2014; Folorunso et al. 2019; Akintelu and Folorunso 2019a; Akintelu and Folorunso 2019b) (Figure 3).

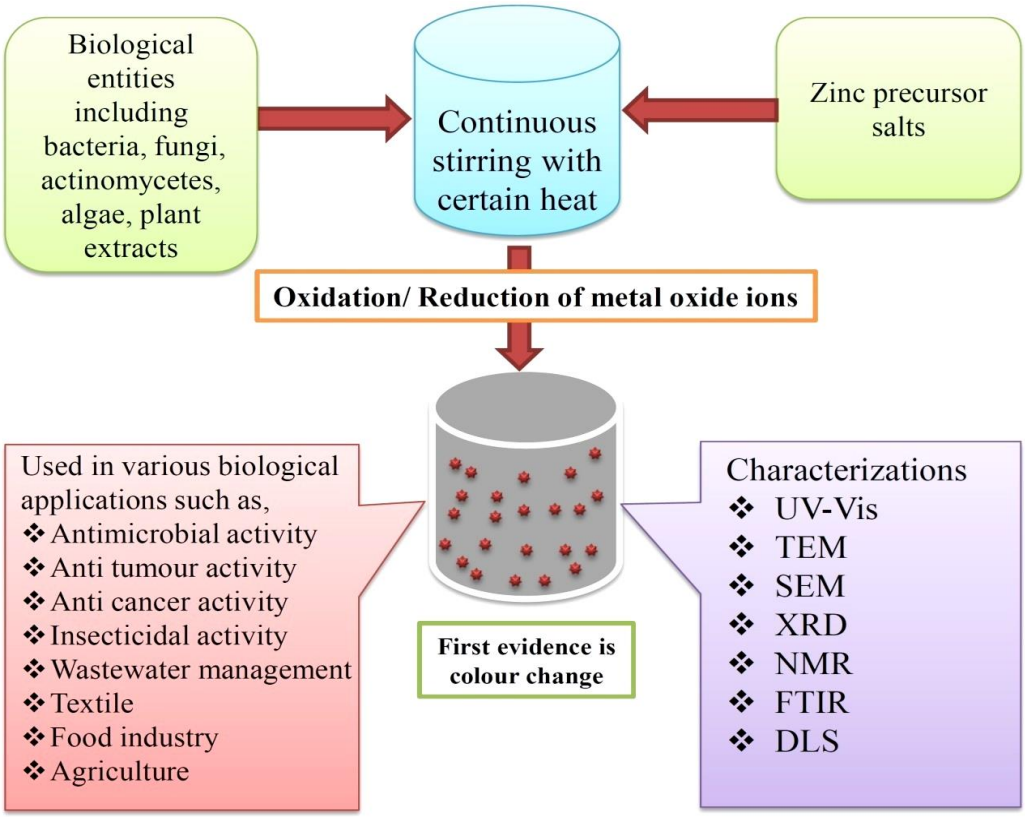

Fig. 2 Flowchart represents the green synthesis of zinc oxide nanoparticles, their potential use and characterization methods

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Bacteria mediated synthesis of zinc oxide nanoparticles

ZnO NPs synthesis using bacterial species gain more attention in past recent years. ZnO nanoparticles were synthesised by *Bacillus licheniformis* through an environment friendly approach showed photo-catalytic and dye degradation activity (Raliya and Tarafdar 2013). Tripathi et al. (2014) describes synthesis of ZnO nanoparticles by using *Rhodococcus pyridinivorans* bacteria. Some study shows the usage of *Aeromonas hydrophila* as reducing agent for synthesis of ZnO NPs (Mehta et al. 2009) (Table 1).

Table 1 Bacterial specimens involved in the synthesis of zinc oxide nanoparticles

Sl. No.	Bacteria Name	Family	Size and Morphology	Application	References
1	*Aeromonas hydrophila*	Aeromonadaceae	57-72 nm, Spherical	Antibacterial and Antifungal activity	Jayaseelana *et al*., 2012
2	*Bacillus megaterium*	Bacillaceae	45-95 nm	Antimicrobial activity	Saravanan *et al.*, 2018
3	*Halomonas elongata* IBRC-M 10214	Halomonadaceae	18.11 nm	Antimicrobial activity	Taran *et al.*, 2018
4	*Sphingobacterium thalpophilum*	Sphingobacteriaceae	40 nm	Antimicrobial activity	Rajabairavi *et al.*, 2017
5	*Staphylococcus aureus*	Staphylococcaceae	10-50 nm	Antimicrobial activity	Rauf *et al.*, 2017

Fungus and yeast mediated synthesis of zinc oxide nanoparticles

Synthesis of ZnO nanoparticles through fungus facilitates large-scale production, highly precise size and shaped NPs (Azizi et al. 2014). Sometimes fungal strains are chosen over bacterial strains because fungal cells contain metal bioaccumulation property and high tolerance (Pati et al. 2014). Some yeast species were also found to be involved in the process of ZnO NPs synthesis (Moghaddam et al. 2017) (Table 2).

Algae mediated synthesis of ZnO nanoparticles

Algae are chlorophyll containing organisms ranges from unicellular to multicellular in nature. Several microalgae draw special attraction because of its capability to degrade toxic metals and also have the capacity to convert them into smaller amount toxic forms (Bird et al. 2015). *Sargassum muticum* and *S. myriocystum* are reported for their role used in ZnO nanoparticles synthesis (Rajiv et al. 2013) (Table 3).

Plant mediated synthesis of ZnO nanoparticles

Plant mediated synthesis of nanoparticles is also termed as 'Green Synthesis' (Shankar et al. 2004). Plants contain a large number of metabolites though their potential impact on synthesis of NPs not fully known and utilized. Using *Vitex negundo* plant extract as reducing agent, Ambika and Sundarajan (2015) reduces zinc nitrate into nano-form which shows antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Kalpana et al. (2017) describes synthesis of ZnO nanoparticles by using *Lagenaria siceraria* pulp extract, its antimicrobial, anti-dandruff and anti-arthritic efficiency. Demissie et al. (2020) reported synthesis of ZnO nanoparticle through *Lippiaadoensis* (Koseret) leaf extract and evaluate its antibacterial activity. Fakhari et al. (2019) used *Laurusnobilis* L. plant extract with zinc acetate and zinc nitrate as Zn precursor. *Cassia fistula* and *Melia azadarach* leaf extracts are used for successful synthesis of ZnO NPs that shows strong antibacterial activity (Naseer et al. 2020) (Table 4).

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Table 2 Fungus and yeast mediated synthesis of zinc oxide nanoparticles

Sl. No.	Fungus Name	Family	Size and Morphology	Application	References
1	*Aspergillus niger*	Trichocomaceae	53-69 nm	Dye degradation and antibacterial activity	Kalpana *et al.*, 2018
2	*Candida albicans*	Saccharomycetaceae	25 nm	Synthesis of steroidal pyridines	Mashrai *et al.*, 2017
3	*Fusarium keratoplasticum* A1- 3	Nectriaceae	10-42 nm	Antibacterial, cytotoxic activity and loaded on textile	Mohamed *et al.*, 2019
4	*Aspergillus niger* G3-1	Trichocomaceae	8-38 nm	Antibacterial cytotoxic activity and loaded on textile	Mohamed *et al.*, 2020
5	*Aspergillus terreus*	Trichocomaceae	10-45 nm	Antibacterial cytotoxic activity, loaded on textile, UV protection	Fouda *et al.*, 2018
6	*Pichia kudriazevii*	Saccharomycetaceae	10-61 nm	Antibacterial & Antioxidant activities	Moghaddam *et al.*, 2017
7	*Aspergillus terreus*	Trichocomaceae	8 nm, Spherical	Catalysts, bio sensing, drug delivery, solar cell, cell-labelling, optoelectronics and imaging	Raliya and Tarafdar, 2014
8	*Pichia kudriavzevii*	Saccharomycetaceae	10-61 nm	Antimicrobial and antioxidant activity	Moghaddam *et al.*, 2017

Table 3 Algae reported for synthesis of zinc oxide nanoparticles

Sl. No.	Algae Name	Family	Size and Morphology	Applications	Reference
1	*Chlorella* extract	Chlorellaceae	20±2.2 nm	Photocatalytic activity	Khalafi *et al.*, 2019
2	*Sargassum muticum*	Sargassaceae	30-57 nm	Supplemental drug in cancer treatments	Sanaeimehr *et al.*, 2018
3	*Chlamydomonas reinhardtii*	Chlamydomonadaceae	55-80 nm	Photocatalytic activity	Rao and Gautam, 2016
4	*Sargassum muticum*	Sargassaceae	30-57 nm	One-pot method for synthesis	Azizi *et al.*, 2014

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Table 4 Plant reported for synthesis of zinc oxide nanoparticles

Sl. No.	Plant Name	Family	Size and Morphology	Applications	Reference
1	*Acalypha fruticosa*	Euphorbiaceae	50 nm; Spherical, hexagonal	Antimicrobial activity	Vijayakumar et al. 2020
2	*Allium cepa*(bulb), *Allium sativum*(bulb), *Petroselinum crispum* (leaves)	*Amaryllidaceae Apiaceae*	70 nm, Hexagonal wurtzite	Photodegradation of methylene blue	Stan et al. 2015
3	*Aloe socotrina*	Asphodelaceae	15-50 nm	Used in Drug delivery	Fahimmunisha et al. 2020
4	*Anisochilus carnosus*	*Lamiaceae*	20-40 nm, Hexagonal wurtzite	Antimicrobial activity	Anbuvannan et al. 2015a
5	*Artocarpus gomezianus*	Moraceae	30-40 nm	Cytotoxicity, antibacterial, and antifungal activities	Anitha et al. 2018
6	*Artocarpus gomezianus*	Moraceae	30-50 nm	Active against urinary tract infection pathogen	Santhosh kumar et al. 2017
7	*Calliandra haematocephala*	Fabaceae	19.45 nm	Photocatalytic dye degradation	Vinayagam et al. 2020
8	*Calotropis gigantea*	Apocynaceae	31 nm; Hexagonal and pyramidal	Nitrite sensing, photocatalytic, and antibacterial activities	Kumar et al. 2020
9	*Celosia argentea*	*Amaranthaceae*	25 nm, Spherical	Antibacterial activity, drug delivery	Vaishnav et al. 2017
10	*Ceropegia candelabrum*	*Apocynaceae*	12-35 nm, Hexagonal	Antibacterial activity	Murali et al. 2017
11	*Couroupita guianensis*	*Lecythidaceae*	57 nm, Hexagonal	Antimicrobial activity	Sathish kumaret al. 2017
12	*Euphorbia Jatropha*	*Euphorbiaceae*	15 nm, Hexagonal	As semiconductor	Geetha et al. 2016
13	*Jacaranda mimosifolia* (flower)	*Bignoniaceae*	2-4 nm, Hexagonal	Antimicrobial activity	Shrma et al. 2016
14	*Limonia acidissima*	*Rutaceae*	12–53 nm, Spherical	Shows antibacterial activity	Patil and Taranath 2016
15	*Olea europaea* (leaf extract)	Oleaceae	40.5-124 nm	Antibacterial activity	Ogunyemi et al. 2019

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16	*Parthenium hysterophorus*	*Asteraceae*	27-84 nm, Spherical and hexagonal	Antifungal activity	Rajiv et al. 2013
17	*Phyllanthus niruri*	*Phyllanthaceae*	25.61 nm, Quasi spherical	Catalytic activity	Anbuvannan et al. 2015
18	*Prosopis julifora*	Fabaceae	31.80-32.39 nm, Irregular	Degradation of methylene blue dye	Sheik Mydeen et al. 2020
19	*Rhamnus virgata*	Rhamnaceae	20 nm	Cytotoxic, antimicrobial, and antioxidant activities	Iqbal et al. 2019
20	*Sedum alfredii*	Crassulaceae	53.7 nm; Hexagonal and pseudo-spherical	As nano-electronics	Qu et al. 2011
21	*Solanum nigrum*	*Solanaceae*	29 nm, Quasi spherical	Antimicrobial activity	Ramesh et al. 2015
22	*Tecoma castanifolia*	Bignoniaceae	70-75 nm	Antioxidant, bactericidal, and anticancer activities	Sharmila et al. 2019
23	*Urtica dioica*	Urticaceae	20-22 nm, Spherical	Antidiabetic activity	Bayrami et al. 2020

Characterization of zinc oxide nanoparticles

UV–Visible Spectrophotometry

UV-Vis spectroscopy is most common instrument used to characterize a newly formed nanoparticles by scanning the synthesized nanoparticles with electromagnetic wave around 200-700 nm (Jamdagni et al. 2016). Distinct peak showed by the formed particles is specific to each nanoparticle. Fakhari et al. (2019) observed characteristic peak around 350 nm for ZnO NPs. This kind of results satisfy the standard pattern of ZnO nanoparticles because all metal oxides show wide band gaps and shorter wavelengths, also if the materials are in nanosize, the wavelengths get more shorter (Naseer et al. 2020). Santoshkumar et al. (2017) reported absorption spectra of newly synthesised ZnO NPs within the wavelength range of 300-500 nm. Karthik et al. (2017) reported absorption peaks ranges between 200-400 nm and also describes amount of plant extract and other external entities affecting the band gap and particle size.

Scanning Electron Microscopy (SEM)

In SEM, high powered electron beam is used for obtaining the data regarding the structure and morphology in micro and nano scale materials (Alejandro et al. 2019). Depending on the high magnification, large field depth and electron density of the surface, SEM images are very relevant for topological assessment of ZnO nanoparticles (Mona et al. 2018). Raut et al. (2015) reported synthesis of hexagonal ZnO nanoparticles using *OcimumTenuiflorum* leaves extract with a size range of 11-25 nm.

Transmission Electron Microscopy (TEM)

TEM characterization is based on the interaction between the highly sophisticated high density electron bean and nano material (Saha et al. 2018). The interaction between the sample and electron beam produces an image by which the size and morphology of nanoparticles can be determined (Ambika et al. 2015; Aljabali et al. 2018). Demissie et al. (2020) reported spherical shaped agglomerated ZnO NPs as observed through TEM. Suresh et al. (2018) reported synthesis ZnO particles using *Costus pictus* D. Don showed variable morphology (hexagonal and spherical in shape). Thi et al. (2020) synthesized ZnO NPs at various temperatures between 400°C and 700°C and showed that with increasing temperature, size of the formed nanoparticles gradually increased.

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Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to examine the surface adsorption of functional groups on biosynthesised ZnO nanoparticles which help for identification of functional groups which are directly or indirectly responsible for the reduction process (Esrat et al. 2018). Earlier research shows that FTIR describes the effectiveness of biological entities in the process of synthesis of ZnO nanoparticles; thus, FTIR characterization unravels the specific role of various biomolecules in formation of ZnO nanoparticles (Feng et al. 2018). Through FTIR evaluation, Fakhari et al. (2019) established the involvement of different phytochemicals like alcohols, phenols, amines, carboxylic acids in ZnO NPs formation and surface stabilization. Happy et al. (2019) reported that phenolic groups, amines, ether carboxylic acid and hydroxyl group act as perfect capping agent for formation of ZnO NPs and are found obtain strong band at 3377.36 for O-H of phenolic group, 1587.42 for C=C stretching of alkyl ethers, 1419.61 for N=O bend of secondary amines, band at 1004.91 for C-O stretch of ether, and 476.42 cm^{-1}band for carboxylic acid and ZnO stretching.

Energy Dispersive X-ray Spectroscopy (EDX)

EDX is very suitable method for analysis of composition of ZnO nanoparticles. For confirming the components, EDX analysis is playing a very prominent role because each element has unique structural properties which lead to distinct peaks on the X-ray spectrum (Taziwa et al. 2017). The purity of synthesised ZnO nanoparticles can be detected through this EDX method (Bala et al. 2015). Nagarajan et al. (2013) prepared ZnO nanoparticles by using seaweeds showed an elemental composition of zinc (52%) and oxygen (48%). Whereas, Abdul Salam *et al*. (2014) reported the presence of 79.33% zinc and 20.67% oxygen in formed ZnO nanoparticles synthesized using *Ocimum basilicum L. var. purpurascens* Benth plant extracts.

X-Ray Diffraction (XRD)

XRD technique is used to determine the crystalline nature of a material (Agarwal et al. 2019). In this process, ZnO nanoparticles are exposed to high energy electron beam which absorbed and diffracted from the nanoparticles surface thereby providing useful data about the structure and morphology (Aldalbahi et al. 2020). Debye-Scherer's equation is used for determination of crystalline structure of ZnO nanoparticles (Rabiei et al. 2020) which is:**$D = k\lambda/\beta\cos\theta$**, where K=Scherer constant (0.9), λ= X-ray wavelength, β= width at half maximum of the diffraction peak, θ = measured Bragg angle and D= structure and size of ZnO nanoparticles. Generally biosynthesized ZnO NPs showed diffraction peaks at 31.61°, 34.26°, 47.37°, 56.40°, 62.68° and 67.72 with corresponds to 100, 002, 101, 102, 110, 103, and 112 reflection planes indicating the purity and crystalline nature of ZnO nanoparticles (Pelicano et al. 2017).

Application of Zinc nanoparticles

ZnO nanoparticles gain prime attention due to their unique properties and versatile application from electric sensors to cancer treatment (Sabir et al. 2014) (Figure 1 & 4). ZnO nanoparticles are generally non-toxic materials, and it can be used in photo-catalytic degradation, substantial materials of environments pollutants (Ryu et al. 2003). Several applications of ZnO nanoparticles are described as follows:

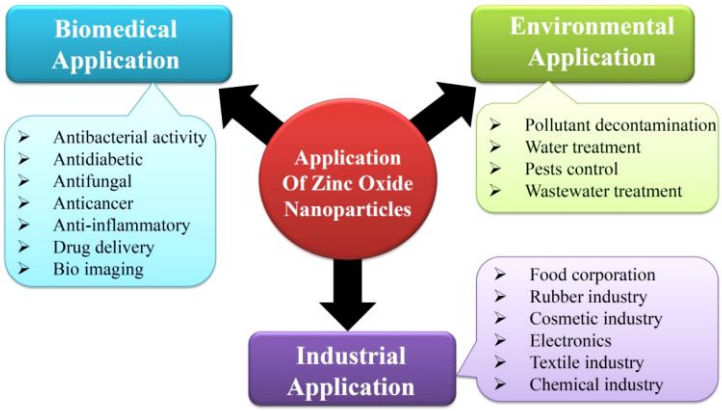

Fig. 3 Major applications of zinc oxide nanoparticles

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Fig. 4 Various applications of zinc oxide nanoparticles

Anticancer activity

Several scientist including Ruenraroengsak et al. (2019), Mahdizadeh et al. (2019), and Hu et al. (2019) reported effectiveness of ZnO nanoparticles against MCF-7 cell lines. Subramaniam et al. (2019) reported effectiveness and use of ZnO nanoparticles against colon cancer. Akintelu and Folorunso, (2020) described that ZnO nanoparticles are able to induce the selective toxicity against only cancerous cells without damaging normal cells.

Antioxidant activity

Antioxidant effect of ZnO nanoparticles is highly reported by several researchers throughout the world. Umar et al. (2019) reported the antioxidant properties of ZnO nanoparticles. Another study showed antioxidant effects of ZnO nanoparticles in monosodium glutamate (MSG)-treated rats (El-Shenawy et al. 2019). Reason behind the antioxidant property of ZnO NPs is electro density to the oxygen molecule (Stan et al. 2016).

Antimicrobial activity

ZnO nanoparticles show strong antimicrobial activity against a wide range of microbes (Akbar et al. 2019). Antimicrobial properties of ZnO nanoparticles showed promising results against several bacterial strains such as *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus, and Salmonella typhimurium* and several fungal species *viz. Aspergillus flavus and Aspergillus fumigatus* (Keerthana and Kumar 2020).

Antidiabetic activity

ZnO nanoparticles show remarkable antidiabetic activity especially in glucose tolerance, improvement and reduction of sugar level, increase insulin level, certain level of reduction in triglyceride levels (Raguraman et al. 2020). Siddiqui et al. (2020) reported very promising results on zinc nanoparticles efficiency against diabetes.

Role of ZnO NPs in plant growth and development

Agriculture is the back bone of any developing economy but in present day it's facing various global challenges such as irregular rainfall, increasing temperature, shortage of agricultural land, pollution, high food demand in comparison to the production etc. It was estimated that world population will be hit 9 billion by the year 2050, so certain efficient

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technologies must have to introduce to develop agricultural sector (Chen and Yada 2011). Continuous use of antibiotics and fungicides in the agricultural sectors leads to the development of multi-drug resistant microbial strains (Sirelkhatim et al. 2015). The application of ZnO nanoparticles works very efficiently against fungi and other microbial infections in both farm animals and crop plants. Application of ZnO NPs on *Arachis hypogea* plant showed increased seed germination; fast stem growth, and enhanced vigour index of the seedlings (Prasad et al. 2012). Another study on *Solanum lycopersicum* showed efficacy ZnO NPs in plant growth improvement, seed germination rate enhancement as well as protein content increment (Singh et al. 2016). Sun et al. (2020) described physiological, transcriptome and metabolic analysis on tomato plant treated with ZnO nanoparticles and reported that ZnO nanoparticles reduced Fe deficiency, minimized oxidative stress and improved nutrient element contents in tomato plant. Some other experiments of ZnO NPs on carrot (*Daucus carota* L.) plants showed positive result on plant growth and yield (Elizabath et al. 2017). Another experiment on ZnO NPs treated tomato plant showed improved antioxidant system and increment in the rate of proline accumulations that provide plants' stability and improved the photosynthetic efficiency (Faizan et al. 2017). Upadhyaya *et al*. (2017) reported that ZnO NPs protects rice plants from ROS damage by improving the antioxidant enzyme activity during the germination. ZnO NPs treatments on *Allium cepa* L. showed better growth, quick flower appearance than the control plant (Laware et al. 2014). Taheri et al. (2015) highlighted that ZnO nanoparticles treated corn (SC704) plants exhibited improved corn growth and yield in poor fertile soil. Phycomolecules coated ZnO nanoparticles treatment on *Gossypium hirsutum* L. plant showed an increase in growth and total biomass (Venkatachalam *et al*. 2017). Yusefi-Tanha *et al*. (2020) applied different concentrations of ZnO nanoparticles on soil-grown soybean (*Glycine max cv. Kowsar*) and reported improvement in antioxidant enzyme system and yield.

Effect of ZnO NPs in plant abiotic stress management

ZnO NPs play important role for minimizing the harmful effect of ROS in cell organelles of plant system and trigger various defence systems by activating cell signalling cascades and by activating or deactivating certain genes (Hancock et al. 2001). ZnO NPs are also very much effective against various abiotic stresses by increasing the activities of several antioxidant enzymes and accumulating osmolytes along with amino acid residues and nutrients during stress condition (Taran et al. 2017; Venkatachalam et al. 2017; Wang et al. 2018)

Drought stress management

Drought stress in both natural and man-made conditions limits crop production and growth. To counter this drought-induce damage in plants, NPs have promising potential. Under stress condition, the stomatal movement is affected by the NPs (Faizanet al. 2020). Taran et al. (2017) reported that foliar application of ZnO NPs on wheat plants reduce the adverse effects of drought stress and improve the growth and yield. Dimkpa *et al.* (2019) published their intensive research on the mitigation of drought stress in *Sorghum bicolor* var. 251 plants through ZnO nanoparticles. Overall results confirmed that ZnO NP treated plants could improve grain yield (22-183%) and enhancement in nitrogen, phosphorous, potassium and zinc nutrient translocation in comparison with the non-treated plants. Another study showed that foliar application of ZnO nanoparticles on wheat plant can increase the yield and also able to mitigate water deficiency stress (Adrees et al. 2021). Dimkpa et al. (2017) reported positive impacts on application of ZnO, B_2O_3, CuO nanoparticles in minimizing drought stress in soybean plants. Drought stress mainly changes sub-cellular structural modification, and shows accumulation of malondialdehyde (MDA) and osmolytes in plant leaves. Application of ZnO nanoparticles induces synthesis of melatonin and activates several antioxidant enzymes to mitigate the drought stress as reported in maize plant (Sun et al. 2020). Semida *et al.* (2021) investigated foliar application of ZnO nanoparticles to promote drought stress tolerance in *Solanum melongena* L. and showed positive results by improving accumulation of micro and macro- nutrients, increasing relative water content (RWC) and alleviating the cell membrane damage. Tewari *et al.* (2019) reported that increased concentration of zinc affects the activity and production of zinc dependent enzymes such as carbonic anhydrase, which directly or indirectly regulates the CO_2 sensing pathway thereby influencing drought tolerance.

Salinity stress management

According to Soliman et al. (2015), ZnO nanoparticles are very much efficient to medicate the salt stress on *Moringa peregrine* plants. Sanaeiostovar *et al*. (2012) used ZnO NPs to increase antioxidant enzyme activity to stabilize the

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stress condition. Alharby *et al*. (2016) reported that use of 15 mg L^{-1} concentration of ZnO nanoparticles be able to mitigate the effect of NaCl toxicity on tomato. Hussein and Baker (2018) utilized zinc NPs to alleviate salinity stress on cotton plants. Foliar application of ZnO nanoparticles on wheat and mustard can able to diminish the harmful effect of the salinity stress (Torabian et al. 2016; Fathi et al. 2016).

Metal stress management

Various reports suggested that ZnO nanoparticles are very much effective against the metal stress (Aravind and Prasad 2005). Venkatachalam et al. (2017) reported that ZnO nanoparticles reduce the toxicity induced by Cd and Pd in *Leucaena leucocephala* seedlings. Garg and Kaur (2013) suggested that the presence of Zn decreased the Cd content on the plant body of *Cajanus cajan,* and enhanced growth, yield and survival of the plants.

Uptake, translocation and accumulation of Zinc nanoparticles in plant system

Plants are one of the most important components of food chain. Uptake, transport and accumulation of nanoparticles in the plant system directly affect the food web (Wang et al. 2013). The mechanism of uptake, translocation and accumulation of nanoparticles in plant system mainly depend on the plant species and the size, configuration and concentration of nanoparticles. The transport of nanoparticles and its adhering property mainly depend on several physical properties such as, van der Waal forces, Brownian motion, gravity, surface tension etc. (Handy et al. 2008). Nanoparticles may dissolve in soil water and dissociate into ions which can move easily through the ion channels (Gupta et al. 2016). Vascular bundle (xylem) acts as most important transporter for distributing and translocation of NPs to the leaves and other terminal parts. Epidermis, cortex, endodermis, cambium, and vascular bundles are mostly interacted place of nanoparticles and accumulate high concentration of nanoparticles than the other plant parts (Faizan et al. 2020). On the other hand, the surface characteristics of nanoparticles are one of the key factors that regulate accumulation, transport and uptake (Nair et al. 2010). López-Moreno et al. (2010) describes accumulation of ZnO NPSs in *Glycine max* seedlings. Another study of zinc nanoparticles uptake through the root system of *Lolium perenne* plant showed promising evidence that the particles

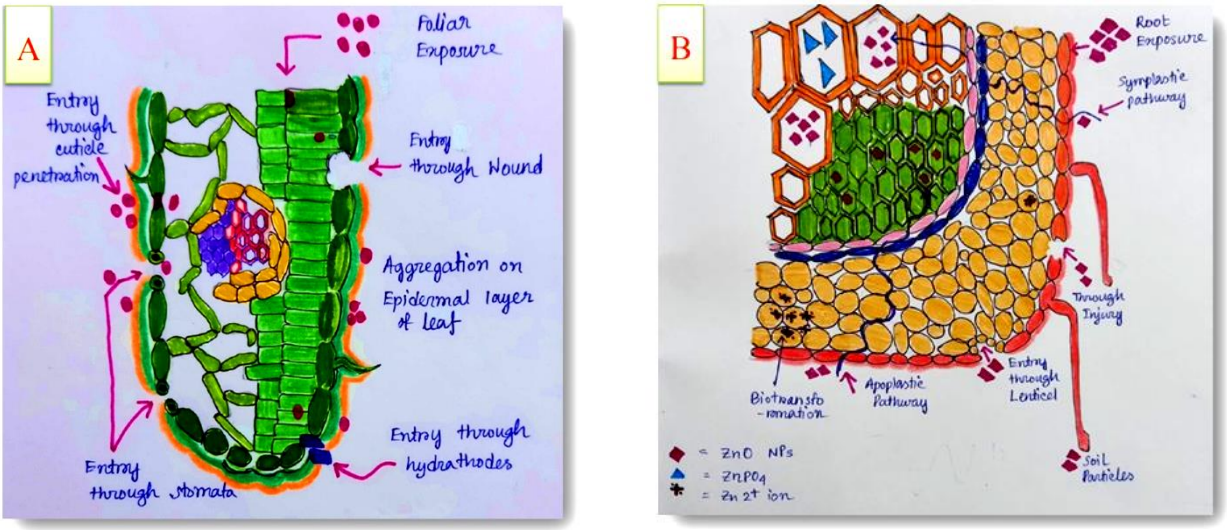

Fig. 5 An overview showing general uptake, translocation and biotransformation process of zinc oxide nanoparticles in plant system. Transverse-section of leaf (A) and root (B) showing entry of nanoparticles

scattered in the apoplast, cytoplasm, epidermal cells and vascular bundle cells (Lin and Xing 2008). Salt et al. (1999) previously described that carboxyl groups play important role in transport and storage of zinc in plant shoot. Lv et al. (2015) studied accumulation and uptake of ZnO NPs in maize (*Zea mays* L. cv. Zhengdan 958) plant and by characterizing through XAS, μ-XRF, SEM, TEM, fluorescence labelling techniques, reported large amount of Zn^{2+} ions uptake and accumulation in the form of $ZnPO_4$. ZnO nanoparticles which adsorbed through the root surface directly affect cell division and elongation. ZnO NPs applied on the leaves may act differently, where they can only enter into the

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plant system through stomata or cuticles and their cellular transport is occurring through the apoplastic and symplastic routes into the vascular bundles of the plant (Figure 5). Leaf morphology, anatomical characteristics, chemical components etc. are essential factors that affect the uptake, accumulation and translocation of nanoparticles in the leaf (Larue et al. 2014). Da Cruz et al. (2019) conducts an experiment to investigate absorption, transportation, and accumulation of ZnO NPs and $ZnSO_4$ on *Phaseolus vulgaris* (L.) plant and reported that smaller nanoparticles (<40 nm) are more likely to be taken up by root system. X-ray fluorescence spectroscopy confirms those root-to-shoot translocations are not very much promising but the radial movement of Zn nanoparticles simultaneously occurs through the xylem transport. Gene coding analysis shows involvement of ZIF1, MTP8, IRT3, HMA2, NRAMP3, NRAMP4, and MTP1 genes for Zn transport in *P. vulgaris* plant system. Al-Salama (2010) describes that when ZnO particles are accumulated in the rhizosphere in higher concentrations, may inhibit the seedling growth in barley plant. Atomic absorption spectroscopy (AAS) is one of the major technologies used for confirmation of nanoparticle uptake and translocation in the plant system (Ju et al. 2019). AAS data showed high accumulation of ZnO NPs in shoot than the root or leaves in rice plant as observed by Afzal et al. (2021). However, for detailed understanding of translocation and accumulation of ZnO nanoparticles in plant system, further investigations are required.

Conclusion and Future prospective

The possibility of synthesizing ZnO NPs using green methods holding a wide range of biological samples has been discussed. With the obtained comprehensive information from this review, it is considered that green synthesis of zinc oxide nanoparticles is much safer and environment friendly than the conventional physical and chemical way of synthesis. In this process, biological entities act as both reducing and capping agents for controlling the synthesis of required size and shape zinc nanoparticles. The generation of reactive oxygen species and easy penetration of ZnO NPs through cell wall has made it a potential therapeutic agent for treating cancer and microbial infections. ZnO NPs would act as nanofertilizer which not only promotes the yield and growth of the plants but also able to mitigate abiotic stresses. Overall, it acts as effective element for sustainable agriculture. Future prospective of biogenic synthesis of zinc oxide nanoparticles include extended laboratory-based work for large-scale production and commercialization, evaluation of toxicity and environmental safety for use in different fields; genome analysis and gene expression studies for understanding the precise mechanism behind the plant growth, development and abiotic stress management.

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