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Deciphering Pollen Parent (*B. nigra*) Compatibility in *B. rapa* Accessions: Pollen–Pistil Interaction Studies Reveal Wide Compatibility in NRCPB-Rapa-8 for Enhanced *B. juncea* Resynthesis

Jyoti Sharma[1,2*], Pooja Garg[1], Shikha Tripathi[1], Sujata Kumari[1], Ranjeet Kushwaha[1], Shiv Shankar Sharma[1], Anamika Kashyap[1], N.C. Gupta[1], Ashish Kumar Gupta[1], M.Z Abdin[2], Ramcharan Bhattacharya[1], Mahesh Rao[1*]

[1] ICAR-National Institute for Plant Biotechnology (ICAR-NIPB), Pusa campus, New Delhi-110012, India

[2] Department of Biotechnology, School of Chemical and Life sciences (SCLS), Jamia Hamdard, Hamdard Nagar, New Delhi- 110062, India

ABSTRACT

Brassica juncea is an amphidiploid (AABB, 2n=36) derived in nature from the hybridization of *B. rapa* (AA, 2n=20) and *B. nigra* (BB, 2n=16), is India's first ranked oilseed crop in terms of production. Its limited genetic diversity due to involvement of only a few parental accession and selections during the developmental process makes it prone to diseases and environmental stresses. Resynthesizing *B. juncea* with diverse progenitor accessions can address this issue but is hindered by embryo abortion and tedious tissue culture for successful wide hybridization processes. At ICAR-NIPB, we identified a novel *B. rapa var.* yellow sarson "NRCPB rapa 8" (IC No. C0623820; INGR17050), enabling *in-vivo* seed development when crossed with *B. nigra* accessions without any tissue culture interventions. At ICAR-NIPB, over the last three years, we crossed three *B. rapa* accessions NRCPB rapa 8 (Br8), rapa 15 (Br4), and pusa gold (Br9) with ten *B. nigra* accessions (Bn). Among the crosses, Br8 showed the highest seed set (16.04%), followed by Br9 (3.35%) and Br4 (0.5%), hybrid formation frequency from F_1 seeds was 84.3%, 31.9% and 0.38% respectively. A total of 13,728 buds were pollinated for seed set and pollen-pistil interaction (P-PI) were studied to address the seed set percentage variation. P-PI were analyzed at 13 time points using aniline blue staining (ABS) method with fluorescence microscope, focusing on pollen germination (Pg), pollen tube growth, and pollen abnormalities. In Br8 and Br9, Pg occurred within 3 hours post-pollination, pollen tube (Pt) reached the mid-style at 6 Hap, and Pt reached ovules by 1 day after pollination (Dap). In contrast, Br4 × Bn exhibited slower pollen tube growth callose deposition, and inhibition of pollen tube growth due to self-incompatibility, confirmed *via* ABS analysis. This research further aims to advance metabolomics and transcriptomics to identify genes involved in wide compatibility in Br8.

Keywords: brassica, resynthesis, genetic diversity, NRCPB rapa 8, pollen-pistil interaction, hybridisation, pollen tube, aniline blue staining

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***Corresponding Authors:**

Jyoti Sharma, M.Z Abdin and Mahesh Rao, ICAR-National Institute for Plant Biotechnology (ICAR-NIPB), Pusa campus, New Delhi-110012, India, Department of Biotechnology, School of Chemical and Life sciences (SCLS), Jamia Hamdard, Hamdard Nagar, New Delhi- 110062, India.
Email: jyoti6335@gmail.com, mzabdin@jamiahamdard.ac.in, mraoicar@gmail.com