Changes in the Weed Seed Bank in Long-Term Establishment Methods Trials under Rice-Wheat Cropping System

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Abstract: The rice–wheat cropping system in the Indo-Gangetic Plains is the backbone of food security in India. In the 1990s, due to the scarcity of resources, the traditional Crop Establishment (CE) method shifted from ConventionalTill Puddle Transplanted Rice (CTPTR) to CT Direct Seeded Rice (CTDSR) and Zero-Till DSR (ZTDSR) in paddy; and in wheat, from Conventional Till Wheat (CTW) to Zero Till Wheat (ZTW), with residue retention in rice (RRR) or in both rice and wheat (RRRW). Shift in CE methods led to change in Weed Seed Bank (WSB) dynamics and ultimately affected the weed management program. After five years of field trials, soil samples were drawn as per 2-factors factorial randomized block design. Factor-I comprised 4-CE methods, whereas factor-II consisted of 3-soil depths (0–10, 10–20 and 20–30 cm). Results showed CTPTR-CTW and ZTDSR-ZTW (RRRW) record the highest seed bank (SB) of grasses, sedges and BLWs as total weeds, in general; and predominant weeds like Echinochloa spp., Ammania baccifera, Commelina benghalensis and Digitaria sanguinalis, in particular. It also showed the higher species richness (DMg) and Shannon–Weaver (H') indices. CTDSR-CTW and CTDSR-ZTW (RRR) show the lowest WSB and at par with Shannon–Weaver (H') index; further, lowest species richness (DMg) under CTDSR-CTW. Species Evenness (J') and Simpson index (λ) vary non-significantly with CE methods. Furthermore, 0–10 cm soil depth showed the highest SB of different category of total weed, predominant weeds as well as higher values of DMg, H', and λ; whereas reverse trend was observed in Whittaker Statistic (βw). Interaction between CE methods and soil depth revealed most of WSB lying on the top layer in case of ZTDSR-ZTW (RRRW) and CTDSR-ZTW (RRR); while CTPTR-CTW showed almost uniform WSB distribution, and in case of CTDSR-CTW, a gradual decrease in WSB with soil depth.

Keywords: population dynamics; tillage systems; vertical distribution; weed emergence; zero tillage

1. Introduction

The rice–wheat cropping system occupies an area of 10.0 million ha in the Indo-Gangetic plains of India and plays a crucial role in the food security of the region [1]. In this region, rice is traditionally established as puddling, followed by hand transplanting of rice seedling and consecutive flooding [2,3]; whereas ploughing is the dominant tillage practice for establishment of
wheat [1,3]. During the 1990s, due to scarcity of water, energy and labor resources vis-à-vis enhanced production cost and diminishing farm profits, initiatives were taken for adoption of resource conservation technologies [4] such as Direct Seeding of Rice (DSR) in Conventional-Till (CT)/Zero-Till (ZT) field, and ZT establishment in case of wheat. Adoption of DSR overcame the problems of labor and water scarcity associated with traditional rice cultivation; further, it matured seven to ten days earlier, facilitating timely sowing of succeeding wheat crop [5]. However, the change in land preparation practices i.e., CT to ZT not only influenced the weed species composition and level of infestation, which subsequently emerged with DSR crop, but also affected the Weed Seed Bank (WSB) dynamics. Actually, in India, in economic terms, weeds in rice field cause a loss of 4420 million USD [6]. This too differs with the Crop Establishment (CE) method; for example, uncontrolled weed growth reduces the yield of transplanted rice by 12 per cent, whereas dry seeded rice sown without soil tillage leads to 98 per cent reduction in crop yield [7].

The WSB, comprised of the weed seeds in different soil profile as well as lying on soil surface, is the principal source of annual weed infestation in field crops [8]. The literature reveals that most of the studies on the influence of crop establishment methods on weed dynamics in rice were based only on the above ground emerged weed flora [9–12], rather than giving due consideration to the WSB dynamics in different soil profiles. In fact, WSB regulates the weed communities and better indicator of long term influence of agronomical practices on weeds rather than the above ground vegetation [13]. The prime objective of weed management in arable crop production is to reduce the WSB in long-term [8]. In general, in the tropical region of India, only limited studies have been conducted on the WSB dynamics of agroecosystems [13].

Further, studies on weed dynamics under differential tillage practices show highly variable results, due to the complex interaction between weed and tillage operations and as the studies are to depend on various factors like weather, duration of experiment, and long-term field history [14]. Studies show that adoption of 4 to 10 years of tillage practice are required to stabilize the yield, weed population and soil characteristics [8]. Therefore, for successful implementation of the integrated weed management practice, studies on long term impact of CE method on WSB is essentially required. Further, the use of indexes of evaluation of the weed population serves as a tool for accessing the impact of agronomic practices [15,16].

Keeping these above facts in view, the present investigation was carried out to study the impact of crop establishment methods on WSB dynamics in rice-wheat cropping system.

2. Materials and Methods

2.1. Site and Soil Information

The field trial was conducted at the Agricultural Research Farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) (128.93 m above mean sea level, 25°15’21” N, 82°59’33” E) situated in the Indo-Gangetic Plains of Uttar Pradesh, India. Being a subtropical climate, May and June in Varanasi are the hottest months of the year with mean temperature ranging from 31 to 36 °C; and January is the coldest month of the year with mean temperature varying from 14 to 17 °C. March to May and December are generally the driest months of the year. The long term average of annual rainfall for this region amounts to 1081.5 mm, of which 944.5 mm (87.33 per cent) is received during the monsoon or rainy season (June to September) and 137.0 mm (12.67 per cent) during post monsoon season or post rainy season. The mean annual Potential Evapo-Transpiration (PET) is 1525 mm.

The soil of experimental field was sandy clay loam in texture (Typic, Ustochrept; Order, Inceptisol), having neutral in soil reaction, low in available nitrogen and organic carbon content, whereas, medium in available P content (Table 1).
Table 1. Physico-chemical properties of the soil of experiment field.

| Soil Properties | Soil Depth 0-10 cm | 10-20 cm | 20-30 cm |
|-----------------|-------------------|----------|----------|
| pH              | 7.41              | 7.51     | 7.62     |
| EC (dSm⁻¹)      | 0.25              | 0.23     | 0.20     |
| Organic Carbon (%) | 0.42           | 0.39     | 0.35     |
| Bulk density (Mg·m⁻³) | 1.40            | 1.42     | 1.45     |
| Available N (kg·ha⁻¹) | 199.60         | 178.70   | 157.81   |
| Available P (kg·ha⁻¹) | 18.55           | 20.10    | 21.83    |

2.2. Soil Sampling and Trial Establishment

The experiment was laid out in the two-factor factorial completely randomized block design, having four crop establishment (CE) methods as the first factor and three soil depth (SD) as the second factor (Table 2), replicated thrice. After the completion of five years of rice-wheat rotation and before the sowing of the sixth cycle of rice crop, soil samples were drawn on 25 June 2017 from variable SD under CE methods, as per the methodology proposed by Forcella et al. [17]. Three soil samples from the central area of each CE methods (4 × 5m), at three different SD were drawn, using a soil auger of 5 cm diameter. The total of thirty-six soil samples taken were placed in the sampling bags, air-dried, and sieved through a 2 mm screen to break up large soil clods. The entire sample, minus large rocks and root fragments, was thinly spread (3–5 cm depth) on the aluminum trays and randomly placed in an open area within one week after the soil collection. The experimental area was covered with a 3 cm mesh-sized nylon net at the top (approximately 2.0 m height). The trays were perforated to facilitate drainage, and soil samples were watered regularly through hose-can to maintain soil moisture content close to the field capacity. This process continued for 100 days until seedling emergence had almost ceased. After the termination of germination, the trays were dried and watered alternatively to stimulate the real field conditions and initiate further emergence. All the emerged weeds seeds have the emergence phase typically in monsoon season i.e., between June to September.

Table 2. Details of treatments.

| Treatments | Abbreviation |
|------------|--------------|
| Crop Establishment Methods (CE) | |
| Conventional Till (CT) Puddle Transplanted Rice—CT wheat: | CTPTR-CTW |
| CT dry Direct Seeded Rice (DSR)—CT Wheat: | CTDSR-CTW |
| CT dry DSR—Zero-Till (ZT) Wheat (residue retention in rice): | CTDSR-ZTW (RRR) |
| ZT DSR—ZT Wheat (residue retention in rice and wheat): | ZTDSR-ZTW (RRRW) |
| Soil Depth (SD) | |
| 0–10 cm | |
| 10–20 cm | |
| 20–30 cm | |

2.3. Observations

For the quantification of WSB, the direct observation of the emerged seedling from each soil sample was applied [18] and the data on emerged seedling were recorded once a week. For the botanical nomenclature of weeds, “Composite List of Weeds” redacted by Weed Science Society of America, available on-line (http://wssa.net/) is used and for those species whose nomenclature is not available in the ‘composite list of weeds’ is verified from the text Walia [19], Xu and Deng [20]. Identified seedlings were counted and removed from the tray, while the seedling of questionable identity was transplanted into pots and grown until their identity verified. Weeds had been classified into the sedges, grasses, broadleaved weeds (BLWs), and values were represented as number m⁻², considering the surface area of the auger.
Different weed diversity indices had been also calculated based on the data of the weed emerged. The Shannon–Weaver index \( (H', [21]) \) was used to measure species richness, evenness and the species diversity in a community \( [H' = -\sum p_i \ln p_i] \). Species richness (Margalef’s diversity index) \( (D_{mg}, [22]) \) was used as a measure of number of different species in a CE method \( (D_{mg} = (S-1)/\ln N) \). Species Evenness (Pielou index) \( (J', [22]) \) describes the evenness or the equitability of the distribution of the numbers of the individuals among the species \( (J' = H'/H'_{max} = H'/\ln S) \). Simpson index \( (\lambda, modified by Barger and Parker [23]) \) measures the probability that two individuals randomly selected from a sampling site will belong to the different species \( (\lambda = 1-\sum p_i^2) \). Whittaker statistic \( (\beta_w, [24]) \) measures the rate of the turnover of the species along a gradient or differences among the individual diversity of CE methods in relation to the total species diversity of experimental field \( [25] \) \( (\beta_m = \gamma/\alpha) \). Where, \( p_i = \) proportion of all individuals which belong to species \( i \) (number of individual of each species \( i/N) \), \( S = total number of species in sample, N = total number of individuals in all species, \gamma = number of species in entire study area, \alpha = number of species per plot within the study area.

2.4. Statistical Analysis

Data collected on weed seeds emerged from soil sample of different CE methods and soil depth were tabulated and statistically analyzed as per the standard statistical procedure [26]. Bartlett’s test was used to test the homogeneity of variance among the treatments. Heterogeneous weed density data were square-root transformed i.e., \( \sqrt{(x + 0.5)} \), prior to analysis to produce a near-normal distribution. However, the original data has been given in the parenthesis for clarity. The significance of the treatment effect was judged with the help of ‘F’ test. The critical difference was worked out at 5 percent level of probability where ‘F’ test was significant.

3. Results

The WSB consisted of the eleven weed species: of which, seven were the broad-leaved weeds (BLWs) (AMBA CAAX, COBE2, ECAL, EUHI, LUHY, and PHNI2); three were grasses (Echinochloa spp., DISA, and DAAE); and one was species of sedge (Sedge, Cyperus spp.) (Table 3). In general, the relative density of WSB showed that BLWs occupy nearly 44–50 per cent population, whereas grasses and sedges occupy 37–44 per cent and 5–18 per cent, respectively.

| Botanical Name                      | US Code/Code | Family      | Common Name           |
|-------------------------------------|--------------|-------------|-----------------------|
| Ammanniabaccifera L. *             | AMBA         | Lythraceae  | Common ammannia       |
| Caesuliaaxillaris Roxb. **          | CAAX         | Asteraceae  | Pink node flower      |
| Commelinabenghalensis L.            | COBE2        | Commelinaceae | Day flower          |
| Cyperusspp.                         |              | Cyperaceae  | Sedge                |
| Dactylocteniumegyptium (L.) Willd  | DAAE         | Poaceae     | Crow foot grass       |
| Digitariasanguinalis (L.) Scop.     | DISA         | Poaceae     | Crab grass            |
| Echinochloa spp.                    |              | Poaceae     | Barnyard grass        |
| Eclipta alba (L.) Hassk.            | ECAL         | Asteraceae  | Eclipta               |
| Euphorbia hirta L.                  | EUHI         | Euphorbiaceae | Spurge             |
| Ludwigialyssapifolia (G. Don) Exell * | LUHY     | Onagraceae  | Water Primrose        |
| Phyllanthusnuriri L.                | PHNI2        | Phyllanthaceae | Niruri            |

Botanical nomenclature and US code (except for * and **) are listed as per “Composite List of Weeds” redacted by Weed Science Society of America and available on-line (http://wssa.net/). * and ** botanical nomenclature listed as per Xu and Deng [20] and Walia [19], respectively and accordingly code was made.
3.1. Effect on Weed Seed Bank (WSB)

3.1.1. Crop Establishment Methods

CE methods significantly affect the WSB of different weeds. *Echinochloa* spp. was the dominant weed in the WSB of different CE methods (29.38%), followed by the AMBA (16.07%), *Cyperus* spp. (11.51%) and others. CTDSR-CTW CE method recorded the lowest seed bank (SB) of the grasses, BLWs, *Echinochloa* spp., AMBA and total weeds (Table 4), statistically at par with CTDSR-ZTW (RRR) CE method. Furthermore, grasses, BLWs, *Echinochloa* spp., AMBA and total WSB were dominant in the ZTDSR-ZTW (RRRW) CE method, at par with the CTPTR-CTW CE method. The sedge (*Cyperus* spp.) and COBE2 SB were dominant in the CTPTR-CTW, but the minimum SB in the CTDSR-CTW CE method. DISA SB recorded the highest and lowest in the ZTDSR-ZTW (RRRW) and CTDSR-CTWCE method respectively. Moreover, the SB of PHNI2 accounts for a considerable amount (9.8%) in WSB but did not vary in the CE methods.
| Treatment          | Grasses       | Sedges       | BLWs         | Total          | Echinochloa spp. | AMBA        | CORE2        | PHNI2      | DISA        |
|--------------------|---------------|--------------|--------------|----------------|-----------------|------------|--------------|------------|-------------|
|                    | Density (n m⁻²) |              |              |                |                 |             |              |            |             |
|                    |               |               |              |                |                 |            |              |            |             |
| CTPTR-CTW          | 68.22 (4865.35) a | 45.01 (2376.10) a | 75.13 (5827.11) a | 112.82 (13068.57) a | 41.27 (2993.23) a, b | 60.70 (3960.17) a | 29.36 (905.18) a | 32.66 (1244.63) | 5.57 (113.15) b, c |
| CTDSR-CTW          | 49.41 (2941.84) b | 23.17 (848.61) b | 52.65 (2998.42) b | 79.38 (6788.87) b | 39.28 (2149.81) b | 25.54 (848.61) c | 6.60 (169.72) b | 20.24 (622.31) b | 0.71 (0.00) c |
| CTDSR-ZTW (RRR)    | 51.33 (3337.86) b | 32.54 (1244.63) a, b | 58.14 (4186.47) b | 86.69 (8788.87) b | 41.51 (2149.81) b | 29.66 (1188.05) b, c | 19.70 (848.61) a | 24.93 (848.61) b | 15.97 (452.59) a, b |
| ZTDSR-ZTW (RRRW)   | 81.98 (8146.64) a | 26.19 (961.76) b | 80.99 (9447.84) a | 121.05 (18556.23) a | 66.85 (5600.81) a | 47.40 (3451.01) a | 21.11 (792.03) a | 28.21 (1923.51) a | 23.93 (1074.90) a |
| CD (p = 0.05)      | 15.44          | 12.84        | 15.44        | 14.23          | 5.17            | 4.85        | 5.18         | 3.89       | 4.89        | 4.25        |

| Soil Depth (SD)    |              |              |              |                |                 |            |              |            |             |
|--------------------|---------------|--------------|--------------|----------------|-----------------|------------|--------------|------------|-------------|
| 0–10 cm            | 88.93 (8613.97) a | 46.35 (2248.81) a | 95.96 (10480.31) a | 140.37 (21342.50) a | 74.96 (6067.55) a | 51.73 (3267.14) a | 34.68 (1357.77) a | 41.83 (2248.81) a | 20.60 (763.75) a |
| 10–20 cm           | 53.30 (3012.56) b | 33.76 (1337.77) b | 62.06 (4030.89) b | 90.31 (8401.22) b | 44.89 (2163.95) b | 38.40 (1569.93) b | 12.75 (721.32) b | 9.68 (381.87) b | 21.75 (721.32) b |
| 20–30 cm           | 45.97 (2842.84) b | 15.08 (466.73) c | 42.15 (2333.67) c | 69.27 (5643.19) c | 36.41 (2163.95) c | 17.77 (848.61) c | 10.15 (509.16) b | 15.95 (509.16) b | 4.35 (84.86) b |
| SEmx               | 4.56          | 3.79         | 4.56         | 4.48          | 4.20            | 4.49        | 3.37         | 4.23       | 3.68        |
| CD (p = 0.05)      | 13.37         | 11.12        | 13.37        | 13.13         | 12.32          | 13.16       | 9.89         | 12.41      | 10.79       |

Data are subjected to square-root transformation ($\sqrt{x + 0.5}$). Data given in parenthesis are original value. Different letters after the mean values within the same category (column) denote significant statistical difference from each other using LSD test at $p = 0.05$, where ‘a’ signifies the highest weed seed bank while ‘c’ for the lowest weed seed bank. S = Significant; NS = Non-significant; CTPTR-CTW, Conventional Till (CT) Puddle Transplanted Rice—CT Wheat (W); CTDSR-CTW, CT dry Direct Seeded Rice (DSR)—CT Wheat; CTDSR-ZTW (RRR), CT dry DSR—Zero-till (ZT) Wheat (residue retention in rice); ZTDSR-ZTW (RRRW), ZT DSR—ZT Wheat (residue retention in rice and wheat).
3.1.2. Soil Depths

Soil depths appreciably affect the WSB of all weeds and record inverse relationship, as the depth increase led to a decrease in the WSB (Table 4). However, the SB of grassy weeds, *Echinochloa* spp., COBE2, PHNI2, and DISA recorded statistically similar SB for soil depth 10–20 cm and 20–30 cm.

3.1.3. Interaction Effect of Crop Establishment Method × Soil Depth

Interaction of crop establishment method (CE) × soil depth (SD) showed significantly highest SB of BLWs, grasses, and total weeds recorded under ZTDSR-ZTW (RRRW) × 0–10 cm (Tables 5 and 6) and was followed by CTDSR-ZTW (RRR). The lowest SB of BLWs, grasses and total weeds, observed in the CTDSR-ZTW (RRR) × 20–30 cm. CTPTR-CTW, showed statistically similar number of BLWs, grasses, and total weeds at all the three soil depths but significant reduction was observed in CTDSR-CTW × 10–20 cm and 20–30 cm, CTDSR-ZTW (RRR) × 10–20 cm and 20–30 cm and ZTDSR-ZTW (RRRW) × 10–20 cm and 20–30 cm.
Table 5. Effects of crop establishment methods and soil depth on the seed bank of BLWs and grassy weeds.

| Crop Establishment Methods | Density of Broad Leaved Weeds (n m\(^{-2}\)) in Different soil Depth | Density of Grasses (n m\(^{-2}\)) in Different Soil Depth |
|---------------------------|----------------------------------------------------------------------------|---------------------------------------------------|
|                           | 0–10 cm                      | 10–20 cm                         | 20–30 cm                  | 0–10 cm                      | 10–20 cm                         | 20–30 cm                  |
| CTPTR-CTW                 | 76.65 (5940.26) b, c         | 77.99 (6109.98) b, c             | 70.74 (5431.09) b, c      | 70.01 (4921.93) b, d, e, f   | 56.52 (3224.71) b, d, e, f       | 78.12 (6449.42) b         |
| CTDSDR-CTW                | 63.44 (4243.04) c, d         | 54.02 (3054.99) c, d, e          | 40.49 (1697.22) d, e, f   | 72.25 (5261.37) b, c         | 45.89 (2206.38) c, d, e, f       | 30.09 (1357.77) f         |
| CTDSDR-ZTW (RRR)         | 91.87 (8486.08) b            | 56.87 (3394.43) c, d, e          | 25.69 (678.89) f          | 81.41 (6788.87) b            | 42.51 (1866.94) d, e, f          | 30.09 (1357.77) f         |
| ZTDSR-ZTW (RRRW)         | 151.90 (23251.87) a          | 59.37 (3564.15) c, d, e          | 31.69 (1527.49) e, f      | 132.06 (17481.33) a          | 68.30 (4752.21) b, c, d, e       | 45.59 (2206.38) d, e, f    |

SEm± 9.12 9.12

CD (\(p = 0.05\)) 26.74 26.74

Data are subjected to square root transformation (\(\sqrt{x + 0.5}\)). Data given in parenthesis is original value. Different letters after the mean values within the same category denote significant statistical difference from each other using LSD test at \(p = 0.05\), where ‘a’ signifies the highest weed seed bank while ‘f’ for the lowest weed seed bank. CTPTR–CTW, Conventional Till (CT) Puddle Transplanted Rice—CT wheat; CTDSDR–CTW, CT dry Direct Seeded Rice (DSR)—CT Wheat; CTDSDR–ZTW (RRR), CT dry DSR—Zero-till (ZT) Wheat (residue retention in rice); ZTDSR–ZTW (RRRW), ZT DSR—ZT Wheat (residue retention in rice and wheat).

Table 6. Effects of crop establishment methods and soil depth on the total weed seed bank.

| Crop Establishment Methods (CE) | Density of Total Weed (n m\(^{-2}\)) in Different Soil Depth |
|--------------------------------|----------------------------------------------------------------|
|                                | 0–10 cm                      | 10–20 cm                         | 20–30 cm                  |
| CTPTR-CTW                      | 118.94 (14526.62) b, c       | 109.32 (12050.24) b, c           | 110.19 (12898.85) b, c    |
| CTDSDR-CTW                     | 105.75 (11371.35) b, c, d    | 75.85 (5770.54) e, f, g          | 56.52 (3224.71) f, g     |
| CTDSDR-ZTW (RRR)              | 131.05 (17311.61) b          | 80.04 (6449.42) d, e, f          | 48.97 (2545.82) g        |
| ZTDSR-ZTW (RRRW)              | 205.74 (42430.41) a          | 96.01 (9334.69) c, d, e          | 61.40 (3903.60) f, g     |

SEm± 8.96 8.96

CD (\(p = 0.05\)) 26.74 26.74

Data are subjected to square root transformation (\(\sqrt{x + 0.5}\)). Data given in parenthesis are original value. Different letters after the mean values denote significant statistical difference from each other using LSD test at \(p = 0.05\), where ‘a’ signifies the highest weed seed bank while ‘g’ for the lowest weed seed bank. CTPTR–CTW, Conventional Till (CT) Puddle Transplanted Rice—CT wheat; CTDSDR–CTW, CT dry Direct Seeded Rice (DSR)—CT Wheat; CTDSDR–ZTW (RRR), CT dry DSR—Zero-till (ZT) Wheat (residue retention in rice); ZTDSR–ZTW (RRRW), ZT DSR—ZT Wheat (residue retention in rice and wheat).
3.1.4. Effect of Crop Establishment Methods on the Vertical Distribution of Weed Seed Bank

The vertical distribution of SB of grasses, sedges, BLWs, and total weeds had been affected by the different CE methods (Figure 1). In CTPTR-CTW, there was almost a uniform distribution of the grasses, BLWs, and total weeds, while the sedges SB observed significant reduction (14.29%) in the 20–30 cm layer. In CTDSR-CTW, the top layer (0–10 cm) contained about half of the total WSB (47–59%) and thereafter gradual decrease in the WSB with respect to the depth, except sedges in which there was an abrupt decrease in SB with depth i.e., from 73.33 per cent in 0–10 cm to 6.67 per cent in 20–30 cm.

Furthermore, in CTDSR-ZTW (RRR) and ZTDSR-ZTW (RRRW) methods, SB of most of grasses (68–72%), BLWs (68–82%), and total weeds (65–76%) was confined to the upper layer of the soil profile (0–10 cm). The bottom layer (20–30 cm) consisted of the minimum grassy weeds (9–14%), BLWs density (5%) and total weeds (7–9%). Conversely, the top layer of soil profile (0–10 cm) consisted of about 54–59% sedges SB, while the subsequent layers, i.e., 10–20 cm and 20–30 cm consisted of 31–35% and 5–6% of sedges density in both the ZT systems (CTDSR-ZTW (RRR) and ZTDSR-ZTW (RRRW)).

Figure 1. Vertical distribution of grasses, sedges and broadleaved weeds (BLWs) in different crop establishment methods of rice-wheat cropping system of Indo-Gangetic Plains. CTPTR-CTW, Conventional Till (CT) Puddle Transplanted Rice—CT wheat; CTDSR-CTW, CT dry Direct Seeded Rice (DSR)—CT Wheat; CTDSR-ZTW (RRR), CT dry DSR—Zero-till (ZT) Wheat (residue retention in rice); ZTDSR-ZTW (RRRW), ZT DSR—ZT Wheat (residue retention in rice and wheat). Note: the percentage was derived by dividing the seed bank at a particular depth to the total seed bank of that category of weed in a particular crop establishment method.
3.2. Effect on Weed Diversity Indices

3.2.1. Crop Establishment Methods

CE methods significantly affected the weed diversity indices mainly, Shannon–Weaver (H’), Species richness (D_{s richness}) and Whittaker Statistic (\beta_W) (Table 7). CTDSR-CTW recorded the lowest H’ and D_{s richness}, while the highest in CTPTR-CTW, statistically at par with CTDSR-ZTW (RRR) and ZTDSR-ZTW (RRRW). Conversely, the highest \beta_W was observed under the CTDSR-CTW and the lowest in CTPTR-CTW which is at par with CTDSR-ZTW (RRR) and ZTDSR-ZTW (RRRW). Apart from this, CE methods did not significantly affect the Species evenness (J’) and Simpson index (\lambda).

3.2.2. Soil Depth

Soil depths also significantly affected all the weed diversity indices. Shannon–Weaver (H’), Simpson index (\lambda) and Species richness (D_{s richness}) follow an inverse relationship with soil depth (Table 7). The Species evenness (J’) firstly increased with an increase in the depth from 0–10 cm to 10–20 cm and then decreased with increase in the depth (i.e., from 10–20 cm to 20–30 cm). Conversely, Whittaker Statistic (\beta_W) value increased with increase in the soil depth, i.e., confirmed direct relationship with soil depth.

Table 7. Effects of crop establishment methods and soil depth on weed diversity indices.

| Treatment                        | Weed Diversity Indices |
|----------------------------------|------------------------|
|                                 | Shannon–Weaver (H’) | Species Evenness (J’) | Simpson Index (\lambda) | Species Richness (D_{s richness}) | Whittaker Statistic (\beta_W) |
| Crop Establishment Methods (CE)  |                        |                        |                         |                                  |                               |
| CTPTR-CTW                        | 1.71 a                 | 0.88                   | 0.78                    | 1.49 a                           | 1.65 b                       |
| CTDSR-CTW                        | 1.33 b                 | 0.93                   | 0.69                    | 0.92 b                           | 2.82 a                       |
| CTDSR-ZTW (RRR)                  | 1.59 a, b              | 0.93                   | 0.75                    | 1.34 a                           | 2.36 a, b                    |
| ZTDSR-ZTW (RRRW)                 | 1.53 a, b              | 0.89                   | 0.72                    | 1.31 a                           | 2.37 a, b                    |
| SEm±                             | 0.09                   | 0.02                   | 0.03                    | 0.10                             | 0.26                         |
| CD (p = 0.05)                    | 0.26                   | NS                     | NS                      | 0.30                             | 0.77                         |
| Soil Depth (SD)                  |                        |                        |                         |                                  |                               |
| 0–10 cm                          | 1.91 a                 | 0.88                   | 0.82 a                  | 1.85 a                           | 1.30 c                       |
| 10–20 cm                         | 1.63 b                 | 0.94                   | 0.78 a                  | 1.24 b                           | 2.03 b                       |
| 20–30 cm                         | 1.08 c                 | 0.90                   | 0.61 b                  | 0.71 c                           | 3.57 a                       |
| SEm±                             | 0.08                   | 0.02                   | 0.02                    | 0.09                             | 0.23                         |
| CD (p = 0.05)                    | 0.22                   | NS                     | 0.07                    | 0.26                             | 0.67                         |
| CE × SD                          | NS                     | NS                     | NS                      | NS                               | NS                           |

Different letters after the mean values within the same category (column) denote significant statistical difference from each other using LSD test at p = 0.05, where ‘a’ signifies the highest while ‘c’ for the lowest. NS = Non-significant; CTPTR-CTW, Conventional Till (CT) Puddle Transplanted Rice—CT wheat; CTDSR-CTW, CT dry Direct Seeded Rice (DSR)—CT Wheat; ZTDSR-ZTW (RRR), CT dry DSR—Zero-till (ZT) Wheat (residue retention in rice); ZTDSR-ZTW (RRRW), ZT DSR—ZT Wheat (residue retention in rice and wheat).

4. Discussion

4.1. Crop Establishment Methods

Change in the crop establishment methods alters the soil ecology, while affecting the soil nutrient, soil structure and temperature, as well as the depth of burial of weed seeds, which ultimately affects the germination of weed species and its composition [27]. In fact, level of soil disturbance affects the weed species richness, abundance and density of weeds [15]. Species diversity within weed communities and the nature of their relationship are of agronomic importance [28].
This study showed that after five years of rice-wheat cropping system trial conducted through variable crop-establishment methods considerably influence the weed density as well as species composition. BLW and grassy weeds were dominant in experimental field: of which, AMBA, COBE2 and PHN2 are the predominant BLWs; and *Echinochloa* spp., DISA are the predominant grasses. It is theoretically presumed that, under ZT system, the weed lying on the surface would promote predator access to the seed and increasing rate of weed seed removal [14]. However, contrary to this hypothesis, double zero-till system of crop establishment method, i.e., ZTDSR-ZTW (RRRW), contains the highest seed bank of grasses, BLWs, and total weed, including weeds like *Echinochloa* spp., AMBA, COBE2, and DISA (Table 4), particularly in the topmost layer of soil profile (Tables 5 and 6 and Figure 1). These results are in conformity with the Cardina et al. [8] and Yenish et al. [29], which also showed higher WSB in ZT system after long-term crop production. Gallandt et al. [30] are also of the opinion that No-Till (NT) system do not enhance the microbial decay of weed seeds; moreover, proportions of dead or decayed seeds are similar under NT and CT system.

Furthermore, in general, PTR provides less conducive condition for germination of weed seeds [9]; interestingly however, this study provides additional information that large number of dormant seeds remains lying in the puddle soil. In fact, CTPTR-CTW system constitutes the higher number of total WSB, this was primarily attributed to higher number of grassy and BLWs seeds, which contributes nearly 82 per cent of the WSB. Within the WSB, the predominant weeds among the BLWs are COBE2, AMBA; and among grasses, it is *Echinochloa* spp. The biology of *Echnichloa* spp. and COBE2 are well studied by many workers, although the biology of the AMBA was not extensively studied. Biology of *E. crusgalli* and *E. colonum* reveal that these weeds are well adapted to wet habitat, produces 2000–4000 seeds per plant, and under ideal conditions, produce millions of seeds per plant [31,32]. These seeds remain dormant in soil for 8–9 years, show staggered germination [32] and undergo dormancy until they receive suitable condition for growth [32,33]. Similarly, COBE2 produces 8000 and 12,000 seed m$^{-2}$ from underground and aerial seeds [34], show strong dormancy and maximum emergence up to the depth of 5 cm and fail to emerge at the depth of 10–15 cm [35]. From the above-mentioned fact, it is clear that the abundance of predominant weeds under CTPTR-CTW it is likely to suppose the inherent biology of these weeds to survive under PTR condition. Secondly, due to repeated puddling operations over the year in CTPTR-CTW, weed seed, particularly grassy weeds, are distributed in the deeper soil horizon and remain unaffected. Moreover, after the harvest of rice, for the sowing of CTW, the tillage operation is performed only up to the depth of 15 cm; thus, seeds lying in surface soil (i.e., only up to 15 cm) are vulnerable to predation by resident insects, rodents, birds and other organisms [36], while the seeds in deeper horizon remain unaffected. Carabid beetles are highly active on the soil surface and may be particularly important as weed seed predators [36,37]. Furthermore, in CTDSP-CTW, in general, the density of all the weeds seeds are reduced, and interestingly the seed of DISA was absent. It might be due to DISA’s natural short-term persistence seed bank. It means seeds remain viable in the soil only for one to five years [38] and are lost thereafter. Due to the absence of the puddling process in CTDSP-CTW, all the weed seeds including DISA, remain lying on the top layer of the soil and are exposed during conventional tillage operation of wheat sowing, thus resulting in rapid loss of the seeds. Punia et al. [39] also noticed higher weed seed density under CTPTR-CTW as compared to ZTR-CTW, CTR-ZTW, ZTR-ZTW and minimum tillage rice (MTR)-ZTW; further, higher numbers of seeds are distributed up to 10 cm soil depth.

CTDSP-CTW and CTDSP-ZTW show similar WSB, this might be due to the fact that tillage operations before the sowing of DSR exhume buried weed seed to the shallow layers, thus forcing the weed seed suffer from harsh atmospheric conditions like high humidity and moist soil condition, and favors the microbial decay of weed seeds, as well as it exposes them to predation; this might be the reasons for the enhanced mortality and reduced density of WSB. Robert [40], Robert and Feast [41] also observed that weed seeds in shallow soil layers or exhumation of the buried seeds to the shallow layers increase the chances of mortality of the weed seeds as compared to the deeper ones. Actually, the burial of weed seeds in deeper layers provide more or less constant soil conditions, and
longevity of these seeds may be more closely related to the adaptations by the seeds to this condition itself [42].

The diversity indices values obtained in our experiment are within the range of those reported previously for various cropping systems [28,43]. Our findings are consistent with the previously reported H' index value that was found to be less than 2.0 [13]. Moreover, after the five years of rotation, the non-significant difference in Species Evenness ($J'$) and Simpson Index ($\lambda$) clearly indicates that, till now, there was no relative dominance of any species as discernable under the different CE methods. At the same time, significantly lower Species richness ($D_{MS}$) as well as Shannon–Weaver ($H'$) indices under CTDSR-CTW, which critically establish the reduced weed diversity under this CE method; this raises a serious concern. Studies showed that more diverse weed community will be less competitive in any crop and at the same time weed diversity is indicative of the wider sustainability of the whole cropping system [44]. In fact, higher biodiversity is a sign of healthy ecosystem, and will not only increase the stability and productivity of the system but also reduce the susceptibility of invasion to the non-native species. In near future the agronomic implications of lower diversity under CTDSR-CTW have led to dominance of a few competitive, highly adapted, widely distributed weed species, having similar resource requirements to the crop [44], that may pose difficulty in its management as compared to the system being dominated by a few problematic weeds [45]. Moreover, under situation of increased population of dominant weed species, the farmers are more reliant on use of fewer selective herbicide, that would lead to increase in selection pressure and development of herbicide resistant dominant weeds [46]. Currently, many of the world’s cropping system showed herbicide resistance, such that as weed diversity has declined [44] that pose threat to the ongoing sustainability of the whole system. Overall, diversity of the crop, cropping system, weeds and weed management practices, result in better exploitation of available resources [45,47].

4.2. Soil Depth

Irrespective of the weed categories and their species, maximum WSB observed at the top 0–10 cm, which gradually reduced with depth. Likewise, weed diversity indices, except $\beta_w$, also record the similar trend. Higher species richness and Shannon–Weaver index in top 0–10 cm indicate more diverse number of weed species exists; further, higher Simpson index in 0–10 and 10–20 cm soil depth signifies the dominance of some of the species in the upper layer as compared to the lower depth. Usually, the weed seeds from plants firstly occupy the upper soil surface during primary dispersal process [48]; later on, through soil turning tillage process, the dormant seeds reach to the deeper layer [49].

4.3. Crop Establishment Method × Soil Depth

Interaction between CE×SD show maximum SB of BLWs and grasses in the top soil layer (0–10 cm) under ZTDSR-ZTW (RRRW), followed by CTDSR-ZTW (RRR). Actually, year-round mechanical churning of soil, from higher to lower manipulation, followed the patterns of ZTDSR-ZTW (RRRW) > CTDSR-ZTW (RRR) > CTDSR-CTW > CTPTR-CTW. The higher churning of the soil is almost equal is the distribution of seeds in different layers; reduction in churning process successively places the seed in the upper soil layer (Figure 1). This is clearly visible at the depth of 20–30 cm, where CTDSR-CTW show lower number of SB of BLWs and grasses as compared to CTPTR-CTW. Conversely, CTPTR-CTW record the highest density of sedges, Shannon–Weaver index and Species richness.

5. Conclusions

Based on this investigation, it can be concluded that after the five years of the crop establishment system, the highest SB of the BLWs, grasses, and total weeds, in general, and Echinochloa spp., AMBA, COBE2, in particular, is recorded under the ZTDSR-ZTW (RRRW), followed by CTPTR-CTW; and the lowest WSB is observed under CTDSR-CTW and CTDSR-ZTW.
(RRR). In fact, CTDSR-CTW show the lower Shannon–Weaver index and species richness, which implicates reduced weed biodiversity under this system which is a matter of great concern, as there is a chance of dominance of a few competitive, highly adapted, weed species that will threaten the sustainability of the system. Further, the SB of grasses, BLWs, sedges, total weeds as well as weed diversity indices, except Whittaker statistics, decreases with increase in the soil depth. This research further finds that, in ZT system, most of the seeds (68–82%) was confined to the topmost layer of the soil profile; while in CT system, there is either uniform distribution of the weed seeds or gradual decrease in the weed seeds with depth. Furthermore, in comparison to CTPTR-CTW system, the CTDSR-CTW and CTDSR-ZTW (RRR) show higher WSB in the upper layer.

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