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Research article

Deciphering the biochemical and functional characterization of rice straw cultivars for industrial applications

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ABSTRACT

As an agricultural state, Haryana (India) produces about six million metric tons (mt) of rice straw every year from rice cultivation. Currently, rice straw is either burned or ploughed into the field without being turned into a functional product. Burning of paddy straw release green house gases and particulate matter (2.5 and 10 μm), which leads to air pollution and considerable loss of soil property *viz.* nutrients, organic matter, productivity and biodiversity, and on and off-farm humans and animals' health. The biochemically and functionally specified potential for optimal alternative use of the rice straw of 13 most widely produced rice varieties from Haryana's eastern and western agro-climate zones was undertaken. Pusa-1401 variety had the highest cellulose (46.55%) and silica content (13.70%), while Pusa-1718 had hemicellulose (28.25%) and lignin (11.60%), respectively. Maximum nitrogen (0.81%), phosphorus (0.32%) and potassium (2.78%) were found in rice variety Pusa-1509, Pusa-1401 and Rice-6129. The findings seemed to be statistically significant ($p < 0.05$). The biochemical profiles of rice straw cultivars were classified into distinct structural groups (C–H alkalanes, O–H alcohol, C=O, C–H alkanes) based on the FTIR spectrum in order to find the best alternative possibilities for bioethanol and compost production. According to the study, these rice straw varieties could be used to make lucrative industrial products.

1. Introduction

India is a rural civilization, known for diverse farming practises according to its agro-climatic zones. Rice and wheat cropping patterns are extensively practised farming systems in the northern parts of India. Rice straw production in India is estimated to be around 126.6 MT, equal to the average harvest index of 0.45 [1]. However, due to a lack of economically feasible rice-straw utilisation choices, farmers in India, particularly in the north-western regions of Punjab, Haryana, and western Uttar Pradesh, have been forced to burn the straw in their fields. Haryana has 1 million hectares of rice cultivation out of a total land area of 4.42 million hectares, with a

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productivity of 3.1 million tonnes per hectare (Indian Council of Agricultural Research). After harvesting the grain, these cereal crops produce a significant amount of residue. Along with the grains, the rice plant produces nearly half of its non-edible biomass as paddy straw, which is either inadequately treated, underutilised, or burned, resulting in environmental challenges such as green house gas emissions.

In Haryana, over 6 million tonnes of rice straw are burned each year. Burning 1 kg of dry rice straw produces nearly 0.7–4.1 g of methane and 0.019–0.057 g of N_2O, as well as other gaseous pollutants like CO_2, SO_2, NO_x, HCl, and, to a lesser extent, VOC (volatile organic compounds), carcinogenic PHA (polycyclic aromatic hydrocarbons), dioxins, and furans, as well as significant plant nutrient and organic matter losses and soil degradation [2]. This is a low-cost, labor-intensive, and easy agri-waste management method. However, it has a significant negative impact on the agro-ecosystem since it produces large volumes of air pollutants in the natural environment, including coarse dust particles (PM10) and fine particles (PM2.5), which cause smog, haze, and smoke [3]. The burning of straw causes total nitrogen and phosphorus loss (25%), potassium (20%), and sulphur losses (60%). Burning has significant impacts on soil parameters such as temperature, pH, humidity, accessible phosphorus, nitrogen, soil organic matter, and loss of nutrients (FAO, 2013). A significant cause of pollution is the burning of 75%–80% of rice straw [4]. India supplies a substantial share of Asia's rice straw, which is roughly 126.6 MT [1]. Rice straw is a holocellulose-rich (cellulose + hemicellulose) lignocellulose waste [5]. Rice straw is classed as non-food, abundantly available, and high in oxalic acid and silicon, contrary to the straws of other cereals, which are widely utilised as fodder [[6,7]]. The management of agri-waste is urgently necessary to curb the menace of burning. Rice straw can be used in biogas, bioethanol, and biodiesel production as an excellent substrate. However, the main hindrances to the economical utilisation of rice straw are recalcitrant cell walls, *i.e.*, lignin and silica. It contains a high content of cellulose, hemicelluloses, as well as lignin, which can be utilised as a substrate for 2nd generation bio ethanol, compost, mushroom, and biochar production [4]. The difficulty of handling a large volume of biomass is a severe risk to the economy and ecology of India. Since the commercial usage of rice straw for farmers in India is not feasible, particularly in the northern regions of Haryana, Punjab, and Uttar Pradesh, they choose to burn it in their fields. It is crucial to discover profitable, socially economical, and environmentally acceptable alternatives, potions, or options for uses of rice straw in this sense. Due to the immense ecological diversity and geographic size of India, rice varieties are indeed issued in specific agro-ecological zones or states. In order to improve output, several kinds of basmati are released each year in the Indian states of Haryana, Punjab, and Uttar Pradesh, which are famous for disease resistant and ecological acceptability. Although

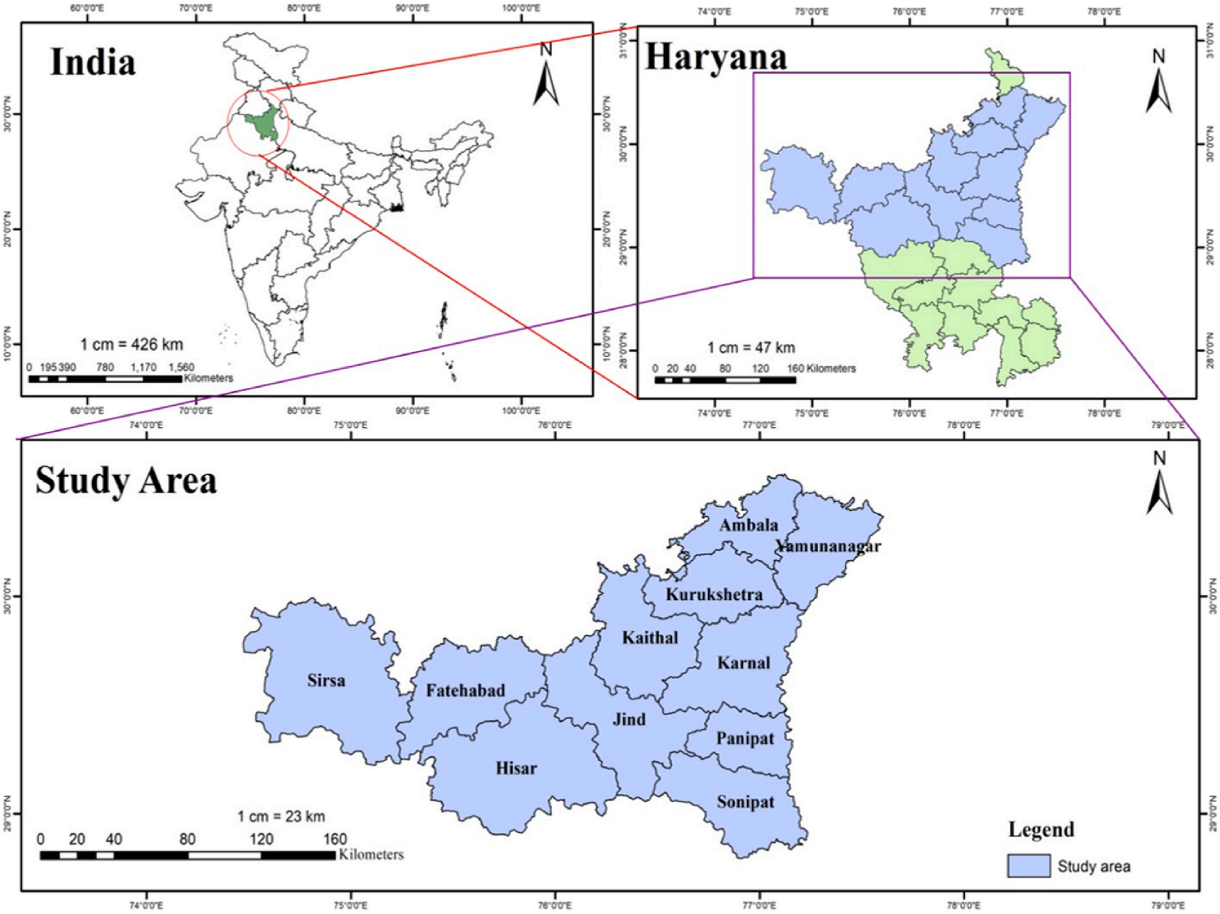

Fig. 1. Area of study.

the farmers in this region still see rice straw as a very significant bioresource, burning straw has already become a detrimental activity. Therefore, it is crucial to identify the rice-straw of common types in this region based on their physio-chemical characteristic properties in order to increase its usability. This kind of data is unavailable and would aid businesses and other stakeholders in choosing particular varieties among them. As a result, the physiochemical and functional groups of the straw from thirteen rice types have been analyzed in order to classify it according to its fitness for various possible applications, including the production of bioethanol and compost.

2. Materials and methods

2.1. Study area

Rice straw was collected from rice fields of 11 districts in Haryana, India, as a lignocellulosic biomass (Fig. 1). Thirteen rice straw cultivars were collected: Pusa-1401, Pusa-1121, Pusa-1718, Pusa-1509, PR-126, PR-114, PR-113, CSR-30, CSR-47, HB-2, Rice-6129, PB-1, and Pusa-441 (Table 1).

The cultivars were picked on the basis of popularity among farmers and customers. Manually rice cultivars were harvested at maturity based on the duration (110–160 days) of all the varieties. The temperature ranged from 20 to 28 °C during harvesting. After harvesting, the grain of rice was separated through thrashing and winnowing, and the straw samples of each cultivar were collected separately. Rice straw samples were dried at 60 °C for 48 h and powdered using a blender fitted with a 2 mm screen and stored for further biochemical and structural groups analysis.

2.2. Biochemical characterization of rice straw

The chemical composition of cell constituents, viz., cellulose, hemicellulose and lignin of rice straw cultivars was determined by the Goering and Van Soest method [8]. The silicon (Si) estimation of rice straw was done by spectrophotometrically [4]. Total nitrogen was estimated by Kjeldahl's system by Foss (Kjeltec™8200). The total phosphorus content was determined by the method of John [9]. The absorbance of blue color was read at 882 nm after 30 min on the UV–Vis. double beam spectrophotometer (Eppendorf Bio spectrometer basic). Total potassium was estimated on a flame photometer by the direct feeding method. The total N, P, and K concentrations in the rice straw were calculated with reference to the standard curve.

2.3. FTIR study of cultivars of rice straw functional groups

The structural changes in functional groups of rice straw cultivars (in triplicate) were analyzed using a Nicolet™ iS50 FTIR (Thermo Fisher Scientific Inc., Waltham, MA, USA) instrument with a KBr disk. The absorption spectra were obtained from (400–4000 cm^{-1}) with a resolution of 2 cm^{-1}.

2.4. Statistical analysis

MS-Excel (Microsoft Office version 2010) was used to statistically analyse the biochemical properties of cultivars of rice straw, including descriptive statistics. The same data set was used to create a heat diagram with SPSS version 26.0 and a dendrogram-clustering with an average linkage between groups of 13 rice straw cultivars produced from hierarchical cluster analysis with SPSS version 26.0 (IBM Corp., 2019, Armonk, NY, USA).

Table 1
Collection of respective rice straw cultivars from different districts of Haryana.

Districts	Variety	Irrigation	Duration (Days)
Sonipat, Jind	Pusa-1121	Low land irrigated	145
	Pusa-1718	Low land irrigated	137
	Pusa-1509	Low land irrigated	115–120
Karnal, Kaithal, Ambala	PR-113	Low land irrigated	142
	PR-114	Low land irrigated	145
	CSR 30	Irrigated	140
	CSR-47	Irrigated	135
Karnal, Panipat	HB-2	Low land irrigated	130–135
	Rice-6129	Up land irrigated	115–120
Yamunagar	PR-126	Irrigated mid early	93–125
	PB-1	Irrigated	130–135
Fatehabad	Pusa-441	Irrigated	110–115
Hisar, Sirsa	Pusa-1401	Irrigated	150–155

3. Results and discussion

3.1. *Biochemical characterization of rice straw*

Initial biochemical characteristics such as cellulose, hemicelluloses, lignin, and silica were used to characterize rice straw from 13 cultivars. The cultivars, as expected, showed variation in lignoellulosic composition. The cellulose, hemicelluloses, lignin, and silica ranged from 37.60 to 46.55%, 22.35–28.25%, 5.60–11.60%, and 9.05–13.70%, with a mean of 42.31, 24.76, 8.78, and 11.1% (Table 2). This result is similar to the results of [4,10] who observed cellulose, hemicelluloses, lignin, and silica percentages ranged from 28.50 to 41.0, 15.3–25.9, 6.2–12.6, and 5–8%, with averages of 36.3, 20.7, 9.8, and 6.7%. On the basis of biochemical properties as depicted in Table 2, rice straw were classified into high, medium, and low ranges of cellulose, hemicellulose, lignin, and silica content using descriptive statistics. The cellulose, hemicelluloses, lignin, and silica in the medium range were 42–44, 24–26, 8–10, and 11–13%, respectively. Variation in the cellulose, hemicellulose, lignin and silica components in 13 cultivars of rice straw is shown in the heat map (Fig. 2). Any numerical value less than the bottom limit of the medium range was classified as low. Likewise, any parameter value that was above the medium range's upper limit was classified as greater.

A dendrogram was used to examine the relatedness of rice straw cultivars based on their biochemical properties (Fig. 3). Pusa-1509 and Pusa-1121 were the most distantly related on the dendrogram, while cultivars, PR-126, Pusa-441, PB-1, HB-2, Pusa-1509, Pusa-1121 and Pusa-1718 formed cluster I; PR-114, CSR-30, CSR-47, Rice-6129, PR113 and Pusa-1401 formed cluster II based on their similarities.

Average linkage treats the distance between two clusters as the average distance between all pairs of samples where one member of a pair belongs to each cluster. The horizontal axis of the dendrogram in this figure depicts the distance or dissimilarity between the clusters. The vertical axis represents the cluster. Pusa-1509 and Pusa-1121 are outliers as they are fused at a much higher distance. Three clusters have three branches that occur at about the same horizontal distance.

3.2. *Macronutrient's concentrations in rice straw*

The results shows that the range of nitrogen, phosphorous, and potassium content varied from 0.32 to 0.81, 0.23–0.32 and 1.38–2.78%, respectively (Fig. 4). Maximum nitrogen content was observed in Pusa-1509 (0.81%), phosphorous in Pusa-1401 (0.32%) and potassium content in Rice-6129 (2.78%). These findings were judged to be statistically significant ($p < 0.05$). The nitrogen content was 1.34, 1.42, and 0.62%, respectively, in cattle dung, biogas slurry and paddy straw [11]. The phosphorous content of different varieties of rice straw ranged from 0.05 to 0.30% [12]. This variation could be due to differences in soil fertility, moisture content, agro-ecological zone, season, crop association and other macro and micro-environmental factors.

3.3. *Structural analysis of rice straw*

Normalization of Fourier transform infrared (FTIR) spectroscopy was carried out to examine functional groups in 13 rice straw cultivars. The structure of cellulose, hemicellulose, and lignin in rice straw was evaluated *via* infrared spectra. An FTIR spectrum was recorded in the range of 4000-400 cm-^{1}. Below 1500 cm^{-1} was the fingerprint region, which is characteristic of the compound. The broad band at 3000–3500 cm-^{1} in the FTIR spectrum indicated the hydroxyl group (O–H) stretching present in cellulose (Fig. 5a–c). The strong absorbance band at 2900 cm-^{1} was due to C–H (alkane) stretching. In the fingerprint region, the characteristic band near 875–930 cm-^{1} was denoted by the glycosidic (1–4) linkage of cellulose. Similarly, the absorption peak at 1720 cm-^{1} was attributed to

Table 2
Biochemical assessment of 13 rice straw cultivars: Descriptive statistics.

Varieties	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Silica (%)
Pusa-1401 (S1)	46.55 ± 0.53	22.35 ± 0.13	9.40 ± 0.55	13.70 ± 0.93
Pusa -1121 (S2)	41.40 ± 0.55	27.30 ± 0.18	11.40 ± 0.58	10.25 ± 0.85
Pusa-1718 (S3)	42.0 ± 0.18	28.25 ± 0.13	11.60 ± 0.77	8.70 ± 0.41
Pusa -1509 (S4)	42.15 ± 0.65	25.65 ± 0.43	7.60 ± 0.48	13.60 ± 0.45
PR – 126 (S5)	39.15 ± 0.56	24.70 ± 0.37	9.40 ± 0.52	12.20 ± 0.48
PR – 114 (S6)	43.15 ± 0.59	25.80 ± 0.37	6.25 ± 0.65	11.15 ± 0.47
PR – 113 (S7)	46.55 ± 0.48	26.20 ± 0.32	5.60 ± 0.41	9.85 ± 0.29
CSR-30 (S8)	42.70 ± 0.71	25.20 ± 0.45	8.05 ± 0.29	9.35 ± 0.59
CSR-47 (S9)	43.45 ± 0.65	22.80 ± 0.45	8.85 ± 0.70	9.05 ± 0.29
HB-2 (S10)	41.15 ± 0.74	23.85 ± 0.43	10.10 ± 0.66	10.95 ± 0.29
Rice-6129 (S11)	45.30 ± 0.33	23.30 ± 0.52	6.90 ± 0.41	11.10 ± 0.97
PB-1 (S12)	37.60 ± 0.45	23.35 ± 0.53	10.00 ± 0.66	13.00 ± 0.45
Pusa-441 (S13)	38.95 ± 0.65	23.15 ± 0.56	9.10 ± 0.52	11.40 ± 0.58
Mean	42.31	24.76	8.78	11.1
Standard error (SE)	0.76	0.50	0.51	0.46
Standard deviation (SD)	2.77	1.82	1.84	1.66
Range –Category –High	>44	>26	>10	>13
Medium	42–44	24–26	8–10	11–13
Low	<42	<24	<8	<11

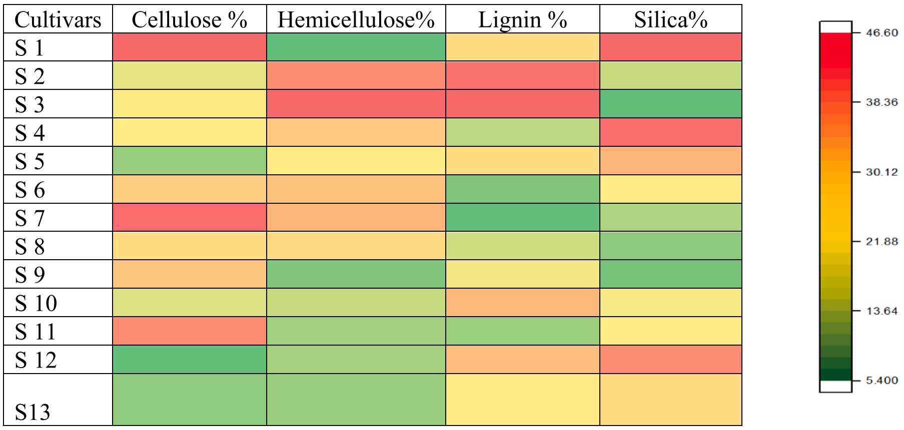

Fig. 2. Heat map of biochemical characteristics of 13 cultivars of rice strawcollected from different districts in Haryana, India.

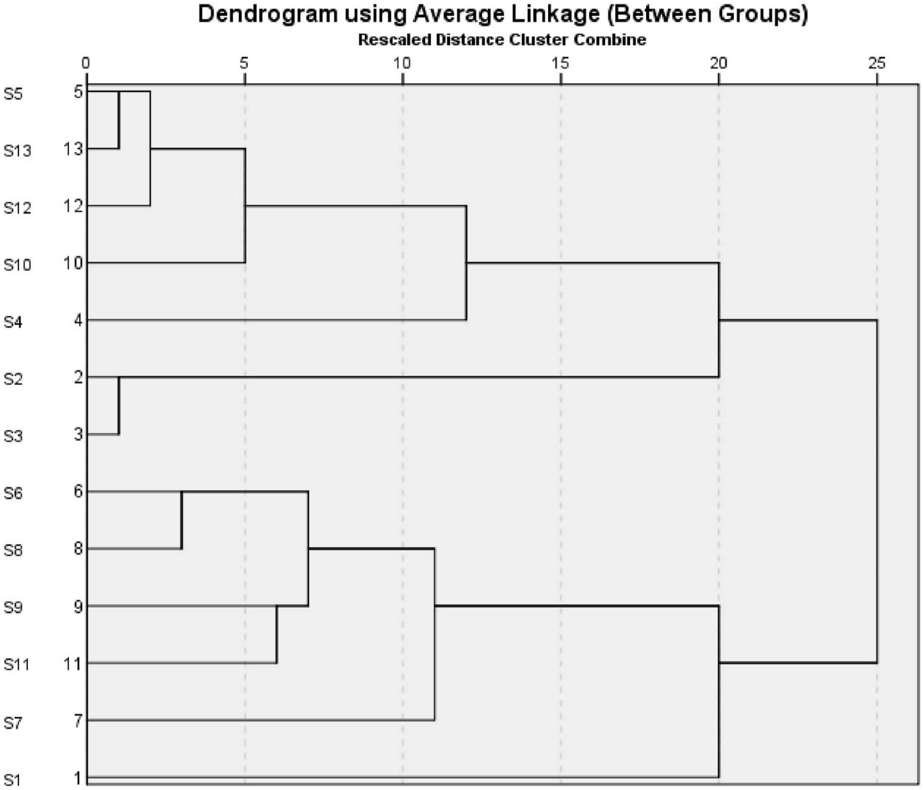

Fig. 3. Based on their biochemical parameters analyzed using Hierarchical cluster analysis, the average linkage (between groups) dendrogram of 13 rice straw cultivars.

the acetyl (C═O) group that is denoted in hemicellulose and lignin content in samples. S9, S10, S11, S12, and S13 samples shown less hemicellulose and lignin content compared over other samples following with heat map (Fig. 2). On the other hand, the band between 1650 and 1515 cm-^{1} has been marked for stretching of the aromatic skeleton (Table 3). The presence of functional groups, the samples S5, S10, S12 and S13 had a low cellulose and hemicellulose content. Considerable amount of cellulose, hemicellulose and lignin was found in S12 and S13. The characteristics of the glycosidic bond (1–4) linkage of cellulose and hemicellulose were assigned to the band at 875–930 cm^{-1}, and similar results were obtained with the band at 899 cm-^{1} for cellulose [13]. The region of hemicellulose and cellulose between 1200 and 1100 cm^{-1} reached its maximum value at 1035 cm-^{1} due to C–O stretching [14]. Like wise, chemical and structural profiling of tetraploid rice straw was documented by Ref. [5]. The crystallinity index of pretreated cellulose was decreased from 63.22% to 57.65% in tetraploid straw as compared to pretreated diploid rice straw. In terms of a more extensive spectroscopy examination, the absorption peak at 341 cm-^{1} (between 3300 and 3500 cm-^{1}) mainly reflects the –OH stretching attributed to alcohol

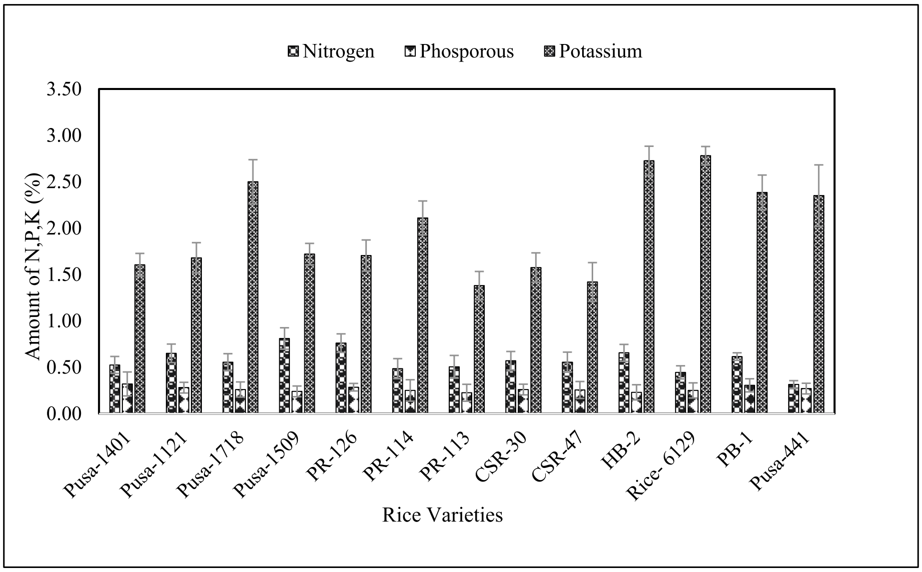

Fig. 4. Determination of nitrogen, phosphorus and potassium content of 13 cultivars of rice straw. NB: bars represent ± standard error.

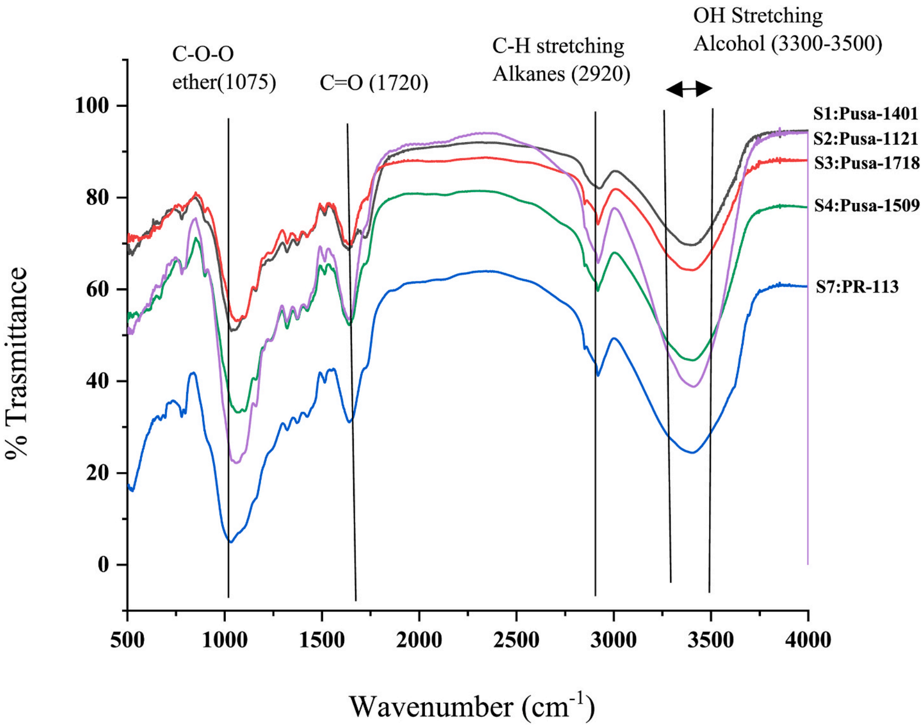

Fig. 5a. FTIR analysis of rice strawcultivars S-1(Pusa-1401), S-2 (Pusa-1121), S-3 (Pusa-1718), S-4 (Pusa-1509), S-7 (PR-113).

skeletal vibrations in cellulose structures. In the literature, several bands have been linked to cellulose and hemicellulose due to their associated functional groups. Normalization of Fourier transform infrared (FTIR) spectroscopy was carried out to evaluate changes in the cell wall and cellulose content of maturing cotton fibres. All single bond-stretching vibration frequencies as well as molecular skeleton vibration frequencies were found in the 910–1300 cm-^{1}region [15].

Based on biochemical features and the presence of functional groups in rice straw, grouping was evaluated for industrial applications such as the generation of bio-ethanol and compost (Table 4). Because these are largely qualitative (or semi-qualitative) attributes, broad groups were identified *via* FTIR observation. However, morphological traits, quantitative biochemical data, and the presence or absence of functional groupings were employed to detect convergence in groupings as mentioned in Table 3.

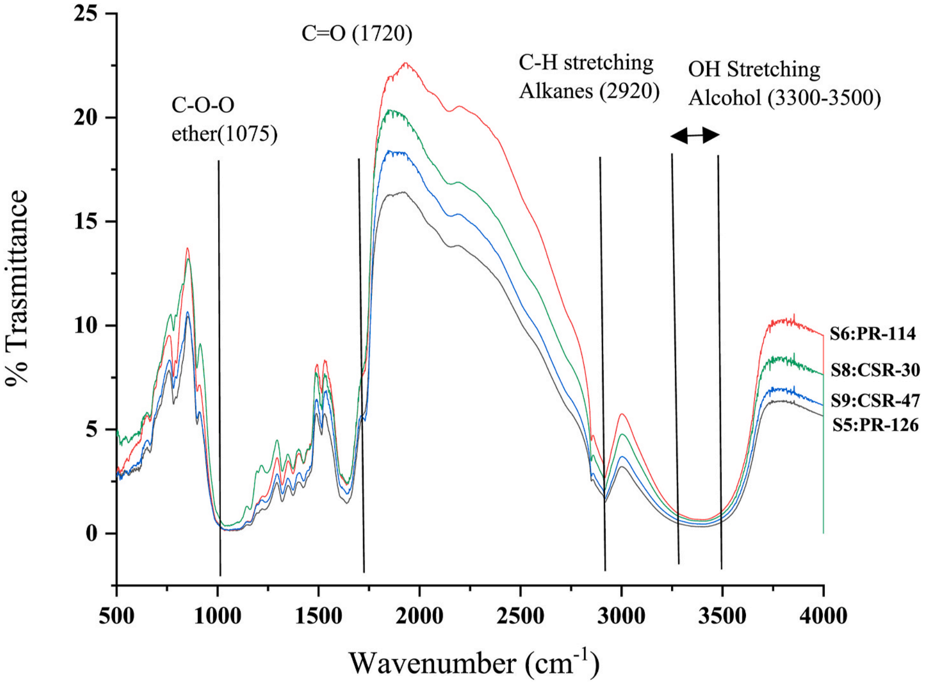

Fig. 5b. FTIR analysis of rice strawcultivars S-5(PR-126), S-6(PR-114), S-8(CSR-30), S-9 (CSR-47).

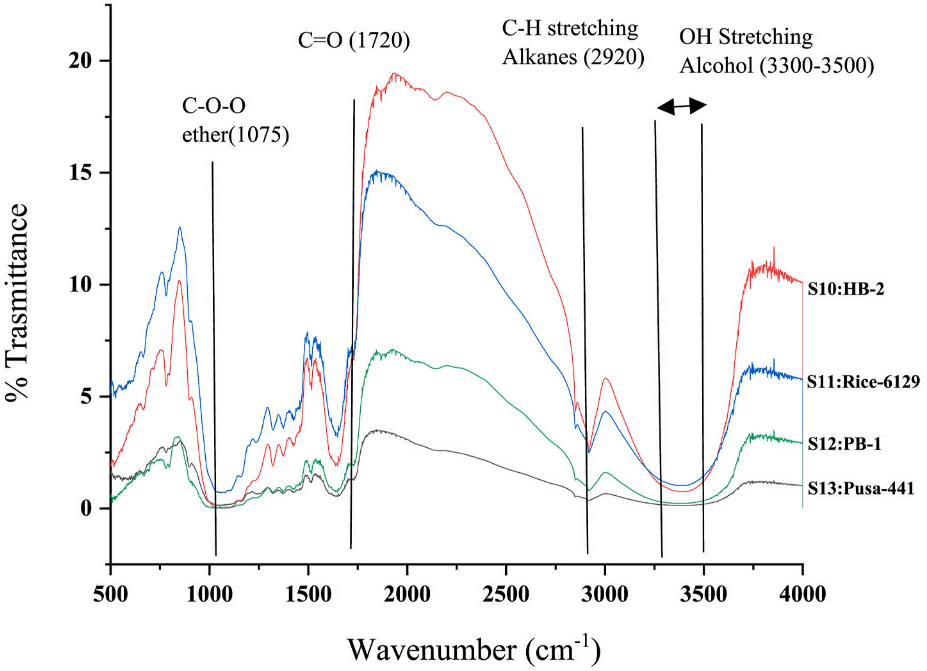

Fig. 5c. FTIR analysis of rice strawcultivars S-10 (HB-2), S-11 (Rice-6129), S-12 (PB-1), S-13 (Pusa-441).

Studies by Ref. [4] reported that bioethanol production mainly depends on the biochemical make-up of the straw, which includes structural or functional arrangements of the straw (cellulose-32-47%, hemicelluloses 19–27%, lignin-5-21%). Cell wall integrity primarily determines the hydrolysis and enzymatic breakdown of cell wall by microorganisms. Based on our findings, rice straw cultivars with higher cellulose, hemicellulose, and low to medium silica and lignin content can be used as substrate at industrial scale to produce bioethanol. FTIR spectra revealed a wide group of rice straw cultivars as functional groups, viz., PR-113, PR-114, Pusa-1509, and Rice-6129, which would be suitable for bioethanol production as they have been assigned glycosidic (875–900 cm-^{1})

Table 3
Functional groups of rice straw cultivars *via* Fourier-transform infrared spectroscopy analysis.

Major components	Wavenumbers/Corresponding peaks (cm^{-1})	Functional groups
Cellulose	2910–2930	C–H alkanes
	875–930	Glyosidic bond
	1075	C–*O*–C
	3300–3500	O–H alcohol
Hemicellulose	1720	C=O
	2920	C–H alkanes
	875–930	Glyosidic bond
Lignin	1720	C=O
	1650–1515	Aromatic

Table 4
Properties of rice straw cultivars for production of value-added products (bioethanol and compost).

Value added products	Properties of rice straw
Bioethanol	High cellulose and hemicellulose Low range to medium silica and lignin C=O,OH, FTIR (cellulose and hemicelluloses group)
Compost	Medium to high cellulose Low range to medium silica and lignin Broad –OH bond (FTIR)

and OH bonds (3300–3500 cm-^{1}), which showed less lignin, considerable amount of cellulose and hemicellulose content. Based on biochemical analysis (cellulose, hemicelluloses, lignin, and silica), it was determined that only three rice straw cultivars, i.e., Pusa-1401, Pusa-1509, PR 113 and Rice-6129, were suitable feedstock or raw material for bioethanol production, with more than 22% hemicelluloses, lower lignin (8%), and 11% silica content, respectively. Among the rice straw cultivars investigated, the PR-113 (S7) was found to be the best for the conversion of bioethanol (Fig. 5b). Many studies have shown that the presence of high levels of cellulose and hemicellulose in rice straw is used as feedstocks for bioethanol [[16,17]]. In rice straw, cellulose and hemicellulose are the easiest to decay, whereas lignin is the most difficult to decay or decompose by microorganisms. As per result, rice cultivars with low lignin concentration should be used as a feedstock for composting. FTIR spectra revealed that a number of our examined cultivars (13 types) have a lower aromatic C=O moiety and a larger C–H stretching in cellulose, making them compostable (microbial decomposition). These were PR-113, PR-114, and Pusa-1121 rice cultivars. Compostable materials with a medium to high cellulose percentage and a low to medium lignin and silica content were preferred.

To classify compost as fertilizer or organic fertilizer, the concentration and availability of nutrients in the compost should be specified. The three nutrients that plants use the most are nitrogen (N), phosphorus (P) and potassium (K). These are the nutrients that are most commonly found in compost [18]. In this direction, Pusa-1509 had the highest nitrogen (0.81%) content, whereas, the phosphorous content in Pusa-1401 (0.32%) and the Rice-6129 variety had the highest potassium content (2.78%). Based on that, we found PR-113, PR-114, Rice-6129, Pusa-1509, and Pusa-1718 were suitable feedstock for compost making. As per literature, no major research has been investigated to quantify the distribution of cellulose, hemicellulose, and lignin content in thirteen rice straw cultivars yet. Based on a combination of FTIR spectroscopic imaging techniques, this work advocated the use of quantitative multivariate spectrum analysis to disclose the biochemical distribution of rice straw.

4. Conclusions

The biochemical analysis of thirteen cultivars of rice straw collected from different areas of Haryana was carried out for analysis of functional group analysis (FTIR), macro nutrients, and cellulose, hemicelluloses, and lignin content in their respective varieties. This was done to find other potential alternative uses like bioethanol and compost as sustainable waste management practices. Results revealed that Pusa-1401 has higher cellulose and silica content than other cultivars. Our observation from biochemical and functional characterisation indicated cultivars, Pusa-1401, PR-113 and Pusa-1718 having higher cellulose and hemicellulose levels, but low lignin and silica could be preferred for bio-ethanol production, while Pusa-1509 (high N) and Rice-6129 high to medium cellulose with low to medium lignin and silica are suitable for compost production. We discovered differences in biochemical characteristics of rice cultivars cultivated in Haryana. The scope of this research was limited to describing the various uses and possibilities for rice straw. The combination of FTIR macro- and micro-spectroscopic imaging techniques might provide a wealth of information for utilising rice straw as a feedstock for bioethanol and compost production as well as minimizing straw burning. Aside from that, rice straw's non-structural content could be evaluated for additional applications. More research is needed, however, to determine the properties of these rice straws for additional applications. These findings will aid in the selection of rice cultivars with high cellulose and hemicellulose content that are ideal for bioethanol and compost production. The wide variability found among the 13 rice straw cultivars evaluated across chemical compositions and macronutrient concentration is a cornerstone for farmers and entrepreneurs, and it could serve as a classic model for paper, pulp production, brick preparation, biofuel, pellet production, animal feed block production, and crop improvement.

Author contribution statement

Kamla Malik and Ajay Sharma: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Dandu Hari karthik, Sunita Rani, Punesh Sangwan and Tanvi Bhatia: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Vijaya Rani, Nisha Arya and Anurag Malik: Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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