CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH

Optimum sampling size for straw and grain yields and plant height in experimental plots of wheat

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Received: 23 December 2021; Accepted: 22 March 2022; doi:10.4067/S0718-58392022000300360

ABSTRACT

Selection of a representative sampling area for accurate and reliable yield evaluation of wheat (*Triticum aestivum* L.) is important for optimizing straw retention in soils and its removal as biofuel feedstock. A field experiment was conducted at four wheat fields in the North China in 2020. Wheat straw and grain yields varied from 359.2-682.8 and 338.5-640.4 g m^{-2}, respectively, and plant height varied from 51.3-59.7 cm across all plots in the four sites. Variation in either relative deviation (RD) or standard error (SE) of straw and grain yield and plant height estimates decreased with the increase in random sampling square (RSS) (one square = approximately 1 m^2) and random sampling plant (RSP) numbers, respectively. Minimum RSS numbers of 3-10 and 1-10 m^2 were needed to satisfy RD less than 5% in two-third of the plots for straw and grain yield estimates, respectively. This suggests that 10 m^2 could be recommended as the minimum RSS number per plot. However, the incidence frequency of RD was 63.75% and 60.00% within the RD interval of 0%-5% for straw and grain yield estimates, respectively, from the RSS number of 10 m^2, indicating that yields from RSSs in field trials are prone to large variations. Therefore, it is strongly recommended to design a large plot as possible and to harvest the whole plot for estimating yields. The threshold RSP number ranged from 14-18 to satisfy RD less than 1.5% and a minimum RSP of 20 plants (including mains and tillers) per plot could be recommended for wheat field experiments.

Key words: Crop residue, optimum sampling square number, random sampling plant number, random sampling square number, *Triticum aestivum*, winter wheat.

INTRODUCTION

Concerns about the environment and sustainable development, coupled with the global abundance of crop residue resources, have recently raised the possibility of using crop residues as a potential feedstock for biofuel production (Lal, 2008; Xie, 2020). However, indiscriminate removal of crop residues from the field may adversely affect the soil, environment, and crop production (Blanco-Canqui and Lal, 2009). The optimal amount of residue that could be removed from the soil as biofuel feedstock depends on the minimum soil retention capacity. The reliability of results and information generated through research largely depends on the quality of the data collected (Knörzer et al., 2013). Fang et al. (2018) established calculation of crop stubble mass amount that could be retained in the soil for nine field crops, based simple linear and power relationships between the post-harvest residue cutting height and stubble mass. Accordingly, accurate and reliable evaluation of crop straw yield is a prerequisite for optimizing straw retention in the soil and its removal from the field. Variation in data could be attributed to controllable variables, such as selection of a representative sampling area (Taye et al., 2000; Knörzer et al., 2013; Zanella et al., 2017).

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world (Lu et al., 2019), and is considered as one of the most major sources of straw for use as biomass feedstock (Garay et al., 2009; U.S. Department of Energy, 2011; Fang et al., 2019). Given the uncertainties in current crop research such as long field experiments cycle, difficulty collecting data, and environmental influence largely, the need for standardizing a suitable sampling size for wheat straw yield in experimental plots is urgent to improve the biomass industry. The accurate estimation of wheat traits in field experiments is difficult, leading to a large number of experimental errors during the sampling process. To obtain accurate data in field experiments, optimizing the sample size is crucial (Brummer et al., 1994). Although innumerable field experiments are conducted globally each year to examine wheat yield, a uniform standard of sampling size for estimating straw yield, grain yield, and plant height in experiment plots is lacking.

The objectives of this study were to determine the optimum sampling area and sampling plant number for estimating wheat straw and grain yields and plant height, respectively. The results of this study will help standardize the sampling size for wheat straw and grain yields and plant height in field experiments.

MATERIALS AND METHODS

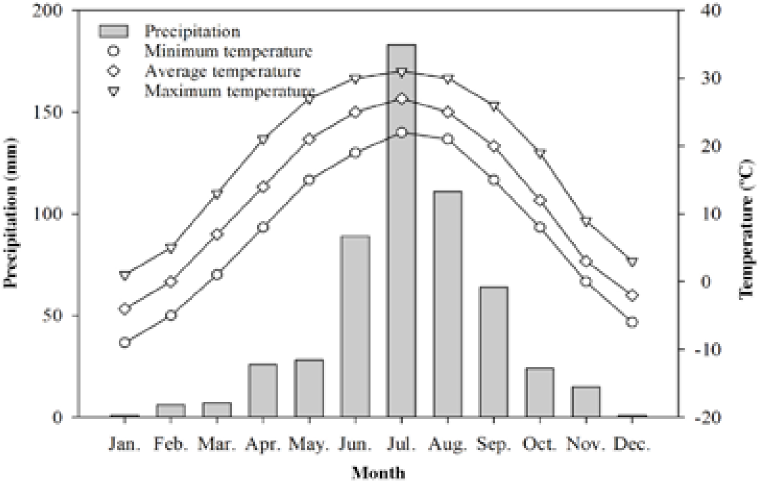
Study sites

This study was conducted in 2020 in a winter wheat (*Triticum aestivum* L.) field used for commercial production. The field is located at the China Agricultural University Experimental Farm in Zhuozhou (CAU-EFZ; 39°47’N, 115°87’E), Hebei province, North China basin. This region has a continental monsoon climate, with cold and dry winters and hot and humid summers. The average annual temperature is 12 °C, and the average annual precipitation is 555 mm, of which approximately 70% falls between June and August. Changes in multi-year average of monthly precipitation and temperature are illustrated in Figure 1. Four sites of winter wheat field were selected from the west and east zones (11 km apart) of the study site. Sites A and B were approximately 230 m apart at the east zone, and sites C and D were approximately 240 m apart at the west zone. Soil samples were collected at a depth of 0-30 cm at each site, and their physical and chemical properties were measured (Table 1). Although the two sites at each site exhibited similar soil properties, large variation was observed between the east and west sites. Compared with the west site, the east site showed higher soil pH, organic matter content, and total N content but lower total P and total K contents. Soil texture is sandy clay loam at sites A and B (east zone) and sandy loam at sites C and D (west zone), according to the USA soil texture classification (Foth, 1990).

Crop management

The local var. Shinong-086 was used for field experiments in all sites. Sowing was conducted on 1 November 2019 at the east site and on 7 November 2019 at the west site. Seeds were sown at a rate of 510 kg ha^{-1} in strips with 15 cm row-to-row spacing. According to local technicians, 81 kg N ha^{-1} and 207 kg P_2O_5 ha^{-1} were used as basal fertilizers before sowing, and 180 kg N ha^{-1}, 45 kg K_2O ha^{-1}, 37.5 kg Ca ha^{-1}, 30 kg Mg ha^{-1}, and 15 kg Zn ha^{-1} were top-dressed as compound

Figure 1. Changes in multi-year average of monthly precipitation and temperature in Zhuozhou, China.



Data were obtained from the National Meteorological Centre (http://www.nmc.cn/).

Table 1. Physical and chemical properties of soil (0-30 cm depth) at the experimental site in Zhuozhou, China in 2020.

Parameter	Site A	Site B	Site C	Site D
Sand, %[a]	52.4	48.7	73.4	65.5
Silt, %[a]	28.9	28.9	19.3	26.2
Clay, %[a]	18.7	22.4	7.3	8.3
pH	7.6	7.6	7.3	7.4
Organic matter, g kg^{-1}	20.0	22.6	13.0	14.4
Total N, g kg^{-1}	1.8	1.9	1.1	1.3
Total P, g kg^{-1}	0.8	0.8	1.1	1.1
Total K, g kg^{-1}	16.1	16.4	19.3	17.5

[a]Soil texture classification was defined according to particle size, as follows: sand, 0.05-2.0 mm; silt, 0.002-0.05 mm; clay, < 0.002 mm.

fertilizers at the regrowth stage in the early spring of 2020. Irrigation was applied five times during the whole crop growth cycle. Weed control was carried out by applying 45 g ha^{-1} tribenuron, 252 g ha^{-1} MCPA-NA, and 150 g ha^{-1} 2,4-D-butyl on 1st April 2020, and pest control was accomplished by applying 900 mL ha^{-1} triazoldone, 750 mL ha^{-1} dimethoate, and 750 g ha^{-1} carbendazim on 10 May 2020.

Experimental design and plant sampling

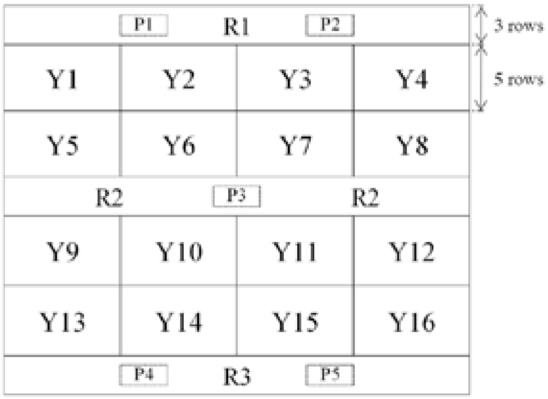
On the physiological maturity dates (7-9 June at the east site, and 11-12 June at the west site), four plots (5 m × 4.8 m) were placed in a square area inside each site. Each plot contained 29 rows, and was divided into 16 yield sampling squares for each (approximate 1 m^2 each) and three remaining parts (Figure 2).

All wheat plants in each sampling square were harvested above the soil surface and wrapped in mesh. The harvested plants were dried under the sun on cement ground, weighed, and then threshed. In the three remaining parts, five points were randomly selected, and the height of 20 successive plants at each point was measured from the soil surface to the tip of the spike (Figure 2). Note that the unit “plants” for measuring the height referred to main stems and tillers together. Thus, a total of 100 plants were used for height measurements per plot. Subsequently, all plants in the three remaining zones were harvested from above the soil surface, wrapped in mesh, and dried under the sun. The dried wheat plants were threshed, and grain and straw weight were measured separately. The grain and straw yields of each plot were calculated as the sum of 16 yield sampling squares and three remaining parts, respectively.

Calculation and statistics

The winter wheat straw yield, grain yield, and plant height data were calculated per plot using Excel 2016. Five combinations of straw yield and grain yield from 1 m^2, 2 m^2, 3 m^2, ... and 16 m^2 (with an interval of 1 m^2) were selected randomly from the 16 yield sampling squares, respectively. The height of 100 plants sampled from five points in each plot was treated as the level of the plot population. Additionally, five combinations of plant height from 5, 10, 15, ..., and 95 plants (with an interval of five plants) were randomly selected from the sample of 100 plants.

Figure 2. Diagrammatic representation of five points (P1-P5, for measuring plant height), 16 yield sampling squares (Y1-Y16), and three remaining parts (R1-R3) in each plot.



The relative deviation (RD, %) and standard error (SE) of each yield or plant height combinations were calculated using Equations 1 and 2, respectively, as follows:

$$\mathrm{RD} = \frac{|\mathrm{SS} - \mathrm{WP}|}{\mathrm{WP}} \times 100 \tag{1}$$

where, SS is the plant height or yield of random sampling square (RSS) combination or random sampling plant (RSP) combination, and WP is the plant height or yield of the whole plot.

$$\mathrm{SE} = \frac{\mathrm{SD}}{\sqrt{n}} \tag{2}$$

where, SD is the standard deviation, and n represents the sample size.

The power function was fitted with changes in the RD and SE of straw yield, grain yield, and plant height estimates with the sample size (i.e., RSP number and RSS number) using Origin 2018 with Equation 3 as follows:

$$y = \frac{a}{x^{b}} \tag{3}$$

where, y is the dependent variable, x is the independent variable for sample size, and a and b are constants.

RESULTS

Performance of wheat yield and plant height

The straw yield, grain yield, and plant height data of winter wheat, representing typical local wheat production data, are summarized in Table 2. The plant height and yield data showed considerable variation in all plots. Plant height (n = 100 plants) varied from 34.8 to 74.5 cm across all plots in the four sites. The whole-plot average straw and grain yield data were the highest in site C (626.6-682.8 and 594.4-640.4 g m^{-2}, respectively) and the lowest in site D (359.2-565.5 and 338.5-534.7 g m^{-2}, respectively) (Table 2).

Table 2. Straw yield, grain yield, and plant height estimates of winter wheat grown in the field.

	Straw yield				Grain yield				Plant height[a]
Site and parameter	Plot 1	Plot 2	Plot 3	Plot 4	Plot 1	Plot 2	Plot 3	Plot 4	Plot 1
	g m^{-2}				g m^{-2}				cm
A Minimum in 16 sample squares[b]	421.4	508.9	314.8	422.8	387.2	496.4	322.6	402.2	38.5
Maximum in 16 sample squares	801.2	686.5	610.6	801.2	669.9	592.4	611.6	684.0	74.5
Average in 16 sample squares	654.0	596.7	467.2	611.7	558.2	550.8	447.8	541.0	57.5
Whole plot (24 m^{2})	631.2	614.8	456.1	567.6	546.4	558.3	434.6	503.3	
Relative deviation (RD)[c], %	3.6	2.9	2.4	7.8	2.2	1.3	3.0	7.5	
B Minimum in 16 sample squares[b]	449.9	335.2	463.1	513.1	344.0	260.0	376.6	509.4	34.8
Maximum in 16 sample squares	840.5	664.6	826.5	799.1	677.6	589.3	674.9	703.6	68.5
Average in 16 sample squares	601.2	481.6	662.1	668.6	471.9	394.6	550.5	595.0	55.2
Whole plot (24 m^{2})	628.0	512.6	680.6	662.9	493.0	425.8	572.7	591.8	
Relative deviation (RD)[c], %	4.3	6.1	2.7	0.8	4.3	7.3	3.9	0.5	
C Minimum in 16 sample squares[b]	497.1	424.8	502.0	553.1	450.0	430.0	529.7	544.2	39.7
Maximum in 16 sample squares	779.8	824.0	820.6	786.6	698.8	658.6	806.5	762.4	72.5
Average in 16 sample squares	640.9	597.2	614.7	683.2	589.6	563.1	607.4	660.3	59.7
Whole plot (24 m^{2})	682.8	626.6	636.1	654.2	625.6	594.4	625.1	640.4	
Relative deviation (RD)[c], %	6.1	4.7	3.4	4.4	5.8	5.3	2.8	3.1	
D Minimum in 16 sample squares[b]	328.1	177.3	333.5	433.0	274.5	173.8	307.2	449.5	35.1
Maximum in 16 sample squares	611.2	487.0	627.9	672.5	523.8	457.0	570.9	621.7	60.8
Average in 16 sample squares	454.0	321.4	461.2	542.4	396.3	300.7	416.7	514.1	51.3
Whole plot (24 m^{2})	463.6	359.2	452.7	565.5	408.4	338.5	411.1	534.7	
Relative deviation (RD)[c], %	2.1	10.5	1.9	4.1	3.0	11.2	1.4	3.8	

[a] The minimum, maximum, and average values of plant number were estimated from 100 plants. The plant number used to measure height included main stems and tillers together.

[b] One sampling square was approximately 1 m^{2} in size.

[c] Relative deviation was calculated as a percentage of the absolute value of the difference between average yield and whole-plot yield divided by the whole-plot yield.

Optimal RSS number for straw yield estimate

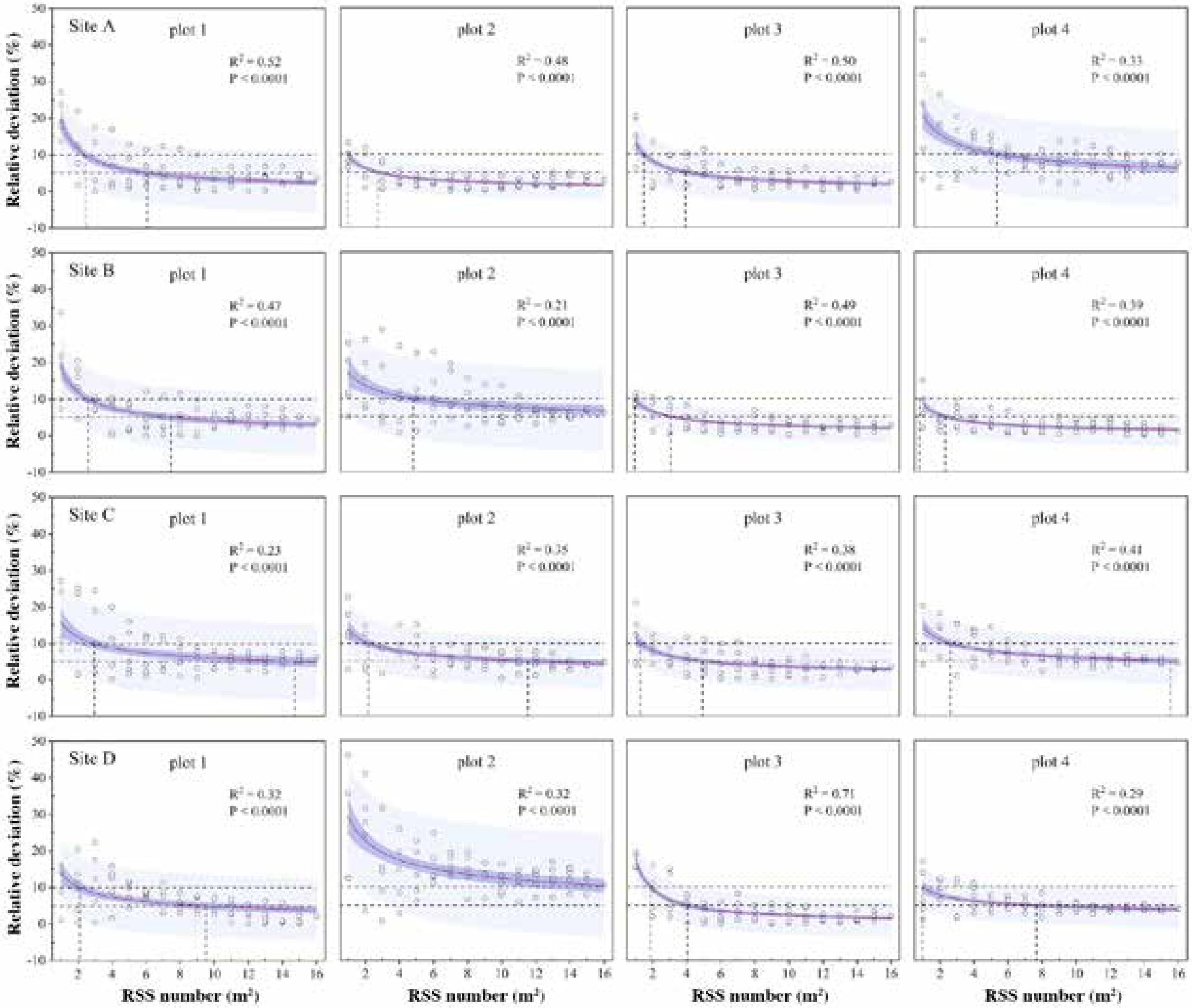
Variation in either RD or SE of straw yield estimate decreased with the increase in RSS number for all plots across the four sites (Figures 3 and 4). Additionally, the RD and SE fitted a power function with highly significant determination coefficients (R^2) ranging from 0.21-0.71 and 0.07-0.67, respectively.

According to the power function between RD and RSS number for straw yield estimate, the threshold RSS number ranged from 1 to 6 m^2 to satisfy the RD of < 10% for all plots (Figure 3). The exception of Plot 2 in site D exhibited threshold RSS number greater than 16 m^2 compared with the whole-plot straw yield. The threshold RSS number ranged from 3 to 10 m^2 to obtain RD < 5% for 10 plots, with an exception of six plots with the RSS number of more than 10 m^2. The SE is the approximate SD of a statistical sample population. The SE of straw yield for the RSS number of 10 m^2 ranged from 17.2 to 35.5 g m^{-2} (Figure 4), which accounted for 3.0% to 6.2% of average straw yield in the whole plot of all sites from.

Optimal RSS number for grain yield estimate

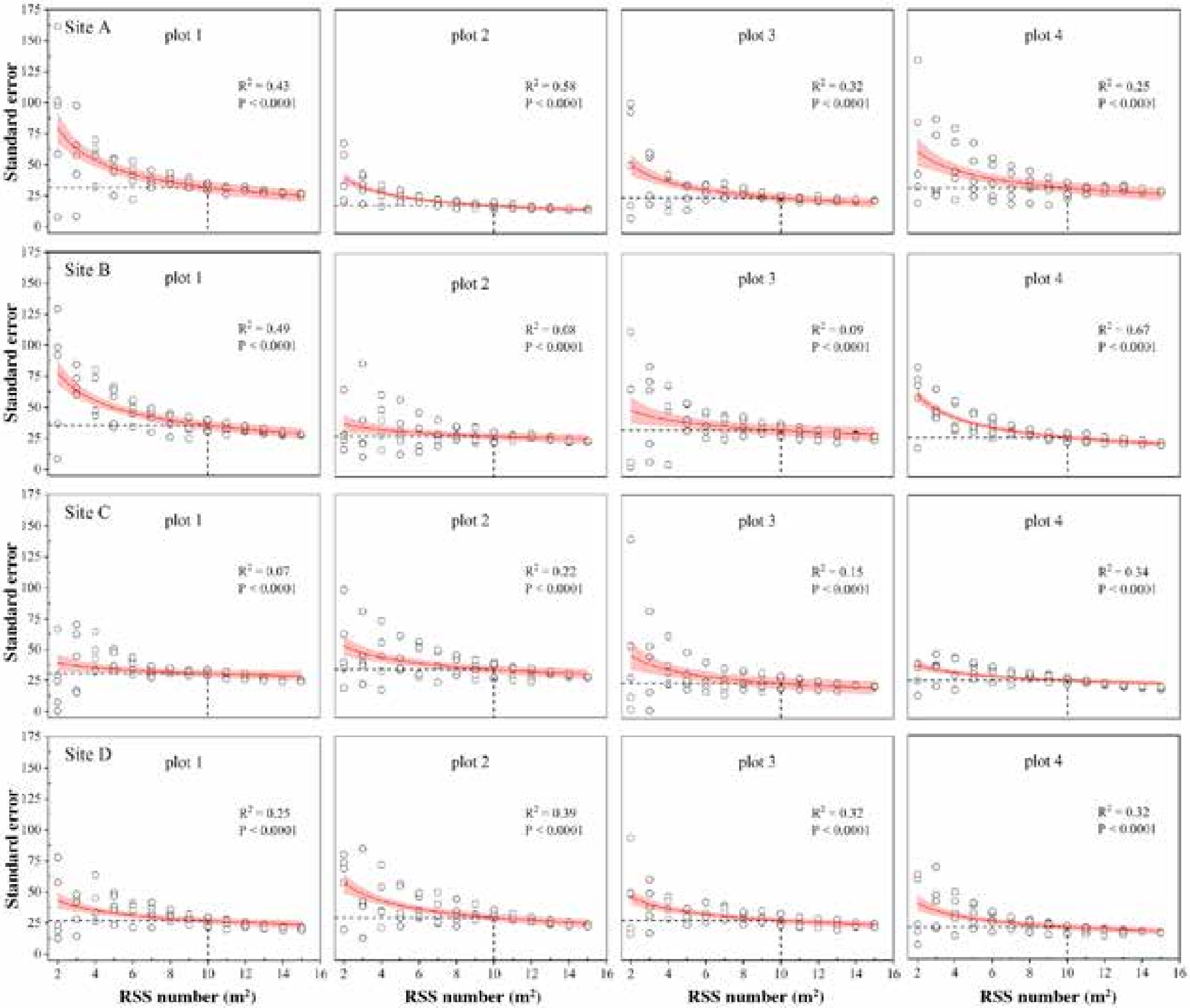
Similar to the straw yield, either the RD or SE of grain yield estimate showed variation, which decreased with the increase in RSS number for all plots (Figures 5 and 6). The RD and SE fitted a power function with highly significant determination coefficients (R^2) ranging from 0.01 to 0.66 and 0.04 to 0.79, respectively.

Figure 3. Change in the relative deviation (RD) of straw yield estimated from five combinations of random sampling square (RSS) numbers, based on the whole-plot straw yield in four plots of four sites.



The power function was fitted for the RD to indicate the optimal number of RSS. Dashed lines indicate the RSS numbers for 5% and 10% RD.

Figure 4. Change in the standard error (SE) of straw yield estimated from five combinations of random sampling square (RSS) numbers in four plots of four sites.



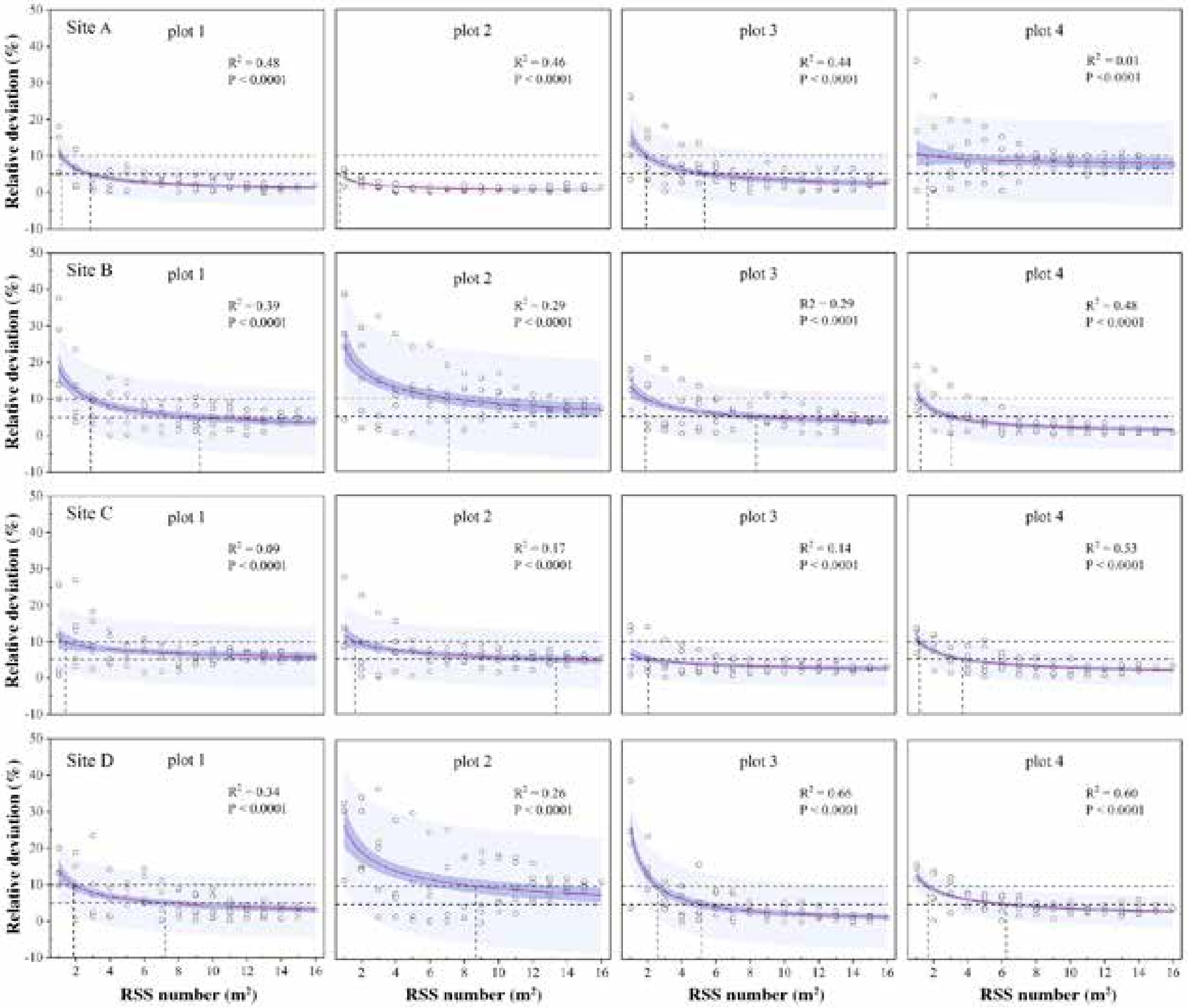
The power function was fitted for the change in SE with sampling square. Dashed lines indicate SE for the RSS number of 10 m^2.

The power function between RD and RSS number for the grain yield estimate showed that the threshold RSS number ranged from 1 to 9 m^2 for RD < 10% of all the plots compared with the whole-plot grain yield (Figure 5). The threshold RSS number ranged from 1 to 10 m^2 to satisfy RD < 5% for 11 plots, with an exception of five plots with an RSS number of more than 10 m^2. The SE of grain yield for the RSS number of 10 m^2 ranged from 9.8 to 31.0 g m^{-2} (Figure 6), which accounted for 2.4% to 8.0% of average grain yield in the whole-plot of all sites.

Optimal RSP number for plant height estimate

Plant height estimate also exhibited the variation of RD and SE decreased with the increase in RSP number for the four sites (Figure 7). The RD fitted a power function with highly significant determination coefficients (R^2) ranging from 0.38 to 0.43. However, the SE fitted a power function with higher determination coefficients ranging from 0.73 to 0.89, based on 100 sample plants as the level of plot population. It was interesting that the variation in plant height estimate was lower than that in yield estimates.

Figure 5. Change in the relative deviation (RD) of grain yield estimated from five combinations of random sampling square (RSS) numbers.



It was based on the whole-plot grain yield. The power function was fitted for the change in RD with RSS number. Dashed lines indicate the RSS numbers for 5% and 10% RD.

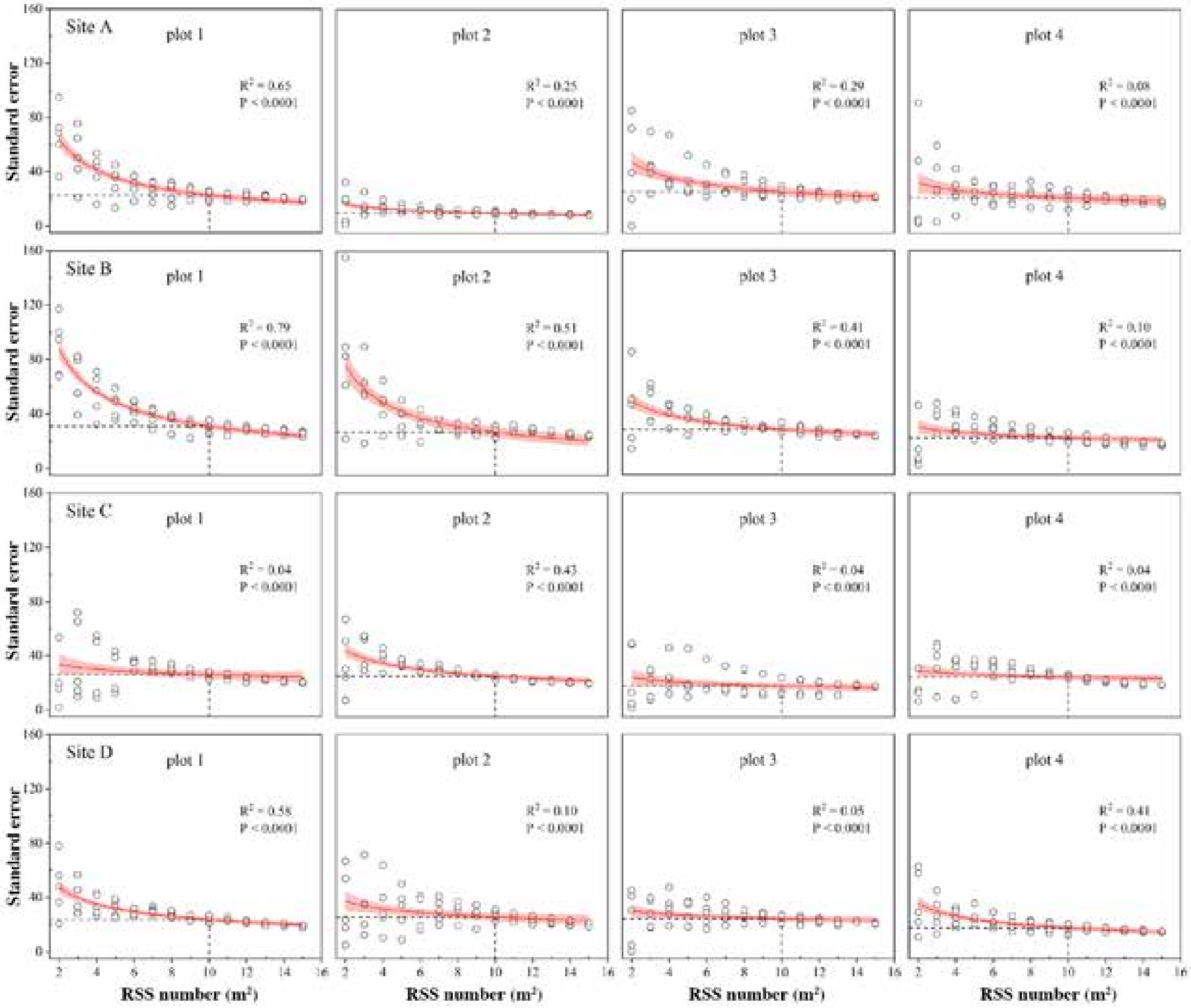
The power function between RD and RSP number for the plant height estimate showed that the threshold RSP number ranged from 28 to 39 for RD < 1% for the four sites compared with the whole-plot plant height, which ranged from 14 to 18 to satisfy RD < 1.5%. The SE of plant height for the RSP number of 20 plants ranged from 1.2 to 1.8 cm (Figure 7), which account for 2.1% to 3.2% of average plant height in the whole plot of all sites.

Incidence frequency of RD of straw and grain yield estimates

The average straw and grain yield estimates of 16 sampling squares (one square = approximately 1 m^2) exhibited an RD ranging from 0.8% to 7.8% and 0.5% to 7.5%, respectively (Table 2), compared with the whole plot (24 m^2 area) for all plots, with an exception of Plot 2 in site D, which showed an RD of 10.5% and 11.2%, respectively. This indicated a large variation in the representativeness of samples for straw and grain yield, even if the sample comprised two-third of the population.

The incidence frequency of RD was 63.75% and 60.00% (within the RD interval of 0%-5%) for straw and grain yield estimates, respectively, from the RSS number of 10 m^2 (Figure 8) but was 75.00% and 68.75% for straw and grain yield estimates, respectively, from the RSS number of 16 m^2. However, even if the RD interval was as wide as 0%-11%, the

Figure 6. Change in the standard error (SE) of grain yield estimated from five combinations of random sampling square (RSS) numbers in four plots of four sites.



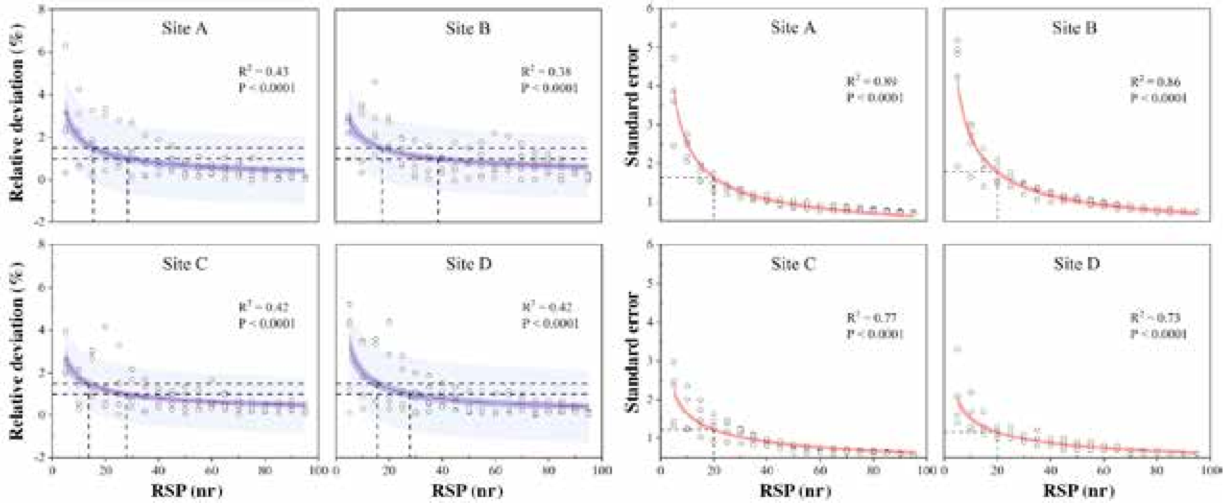
The power function was fitted for the changed in SE with RSS number. Dashed lines indicate SE for the RSS number of 10 m^2.

incidence frequency was 100.00% and 93.75% for the straw and grain yield estimates, respectively, from the RSS number of 16 m^2, which represent two-thirds of the whole plot area (24 m^2). These results indicate that straw and grain yield data obtained from RSSs in field trials are prone to large variations due to uncontrolled conditions.

DISCUSSION

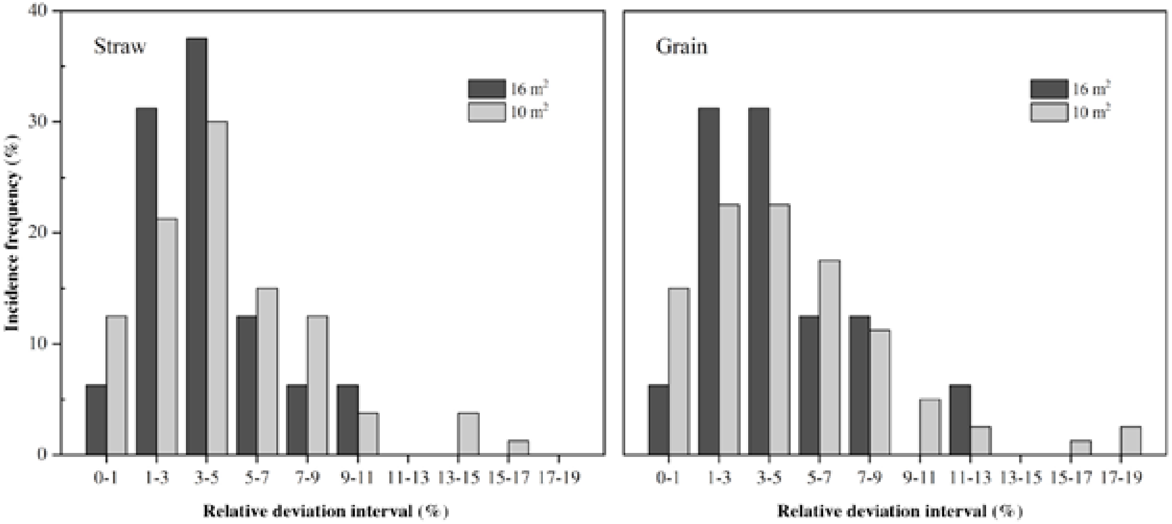
As expected, variation in either the RD or SE of straw yield, grain yield, and plant height estimates decreased with the increase in RSS and RSP numbers. To estimate straw and grain yields, the minimum RSS number ranged from 1 to 6 and 1 to 9 m^2, respectively, to satisfy RD less than 10% for all plots, and from 3 to 10 and 1 to 10 m^2, respectively, to satisfy RD less than 5% in two-third of the plots. Because the RD of 5% would be much better for obtaining a more accurate estimate, the minimum RSS number of 10 m^2 per plot could be recommended for the field experiments of wheat. It was difficult to find relevant literature on wheat. However, a few studies have been conducted on other crops, and their findings were in

Figure 7. Change in the relative deviation (RD) and standard error (SE) of plant height estimated from five combinations of random sampling plant (RSP) number.



It was based on 100 sample plants as the level of plot population in four sites. The number of plants selected for height measurements included the main stems as well as tillers. The power function was fitted for the change in RD and SE with RSP number. Dashed lines indicate the RSP number for 1% and 1.5% RD and the SE for the RSP number of 20 plants.

Figure 8. Changes in the incidence frequency (RD) interval (%) for straw and grain yield estimates from the random sampling square (RSS) number of 10 m^2 and 16 m^2.



The RD for the RSS number of 10 m^2 was based on the fitted power function in Figure 3 and Figure 5, and RD for the RSS number of 16 m^2 was based on the fitted power function in Table 2.

agreement with the results of the current study. Iqbal et al. (2019) reported a sampling area of 9 m^2, under the limit of 90% variance reduction for the DM yield of willow as a biomass crop in south-west Germany. Vallejo and Mendoza (1992) presented an optimal plot size of 5-18 m^2 for sweet potato in Peru. According to the Ministry of Agriculture and Rural Affairs of China, the plot area was suggested as 13.3 m^2 for regional trails of most field crops (NY/T1301, 2007). These findings were in agreement with the results of the current study.

A few studies exhibited findings obviously lower optimal plot size of wheat or other crops than this study. Shah et al. (2017) reported a suitable plot size of 3.05 m × 1.22 m (3.72 m^2) for experimental purposes of wheat yield was found most suitable in Khyber-Pakhtunkhwa, Pakistan. Based on a field trial, a plot area of 5.0-6.5 m^2 was recommended for cabbage in India (Singh, 1989). Knörzer et al. (2013) documented that a cutting regime of more than 5.6 m^2 would be advisable for miscanthus, but an area of 3 m^2 is sufficient to eliminate approximately 90% of the variance. According to field experimental tests in Universidade Federal de Viçosa, Brazil, the plot optimal area was 4.5-5.7 m^2 and recommended for taro (Miyasaka et al., 2013; Silva et al., 2019). The most appropriate optimum plot size was recommended to use 3.9 to 4.8 m^2 (13 to 16 plants) per plot for sweet potatoes in Brazil based on dish-segmented models (Humada González et al., 2018).

However, according to this study, variation in the representativeness of straw and grain yield estimates from RSSs was higher than that of plant height from RSPs per plot. Within the RD interval of 0%-11%, the incidence frequency of RD was 100.00% and 93.75% for the straw and grain yield estimates, respectively, from the RSS number of 16 m^2, which represents two-thirds of the whole plot area. This was due to a large variation in a plot of field tests because of soil and water heterogeneity (Wang et al., 2017). A previous study reported 16 m^2 as the optimal sampling area for potato (Oliveira and Estefanel, 1995) in Santa Maria, Brazil. Masood et al. (2012) also reported the optimum plot size for paddy yield trial was estimated to be 6 m × 12 m (72 m^2) in Kala Shah Kaku, Lahore, Pakistan, based on the coefficient of variation. Therefore, it is strongly recommended to design a large plot as possible and to harvest the whole plot for straw and grain yield on the maturity date.

The threshold RSP number ranged from 28 to 39 plants to satisfy RD less than 1%, and from 14 to 18 to satisfy RD less than 1.5%. Therefore, a minimum RSP number of 20 plants per plot could be recommended for wheat field experiments. This is in agreement with a previous report, which recommended 10-20 plants as the optimized plant number for height evaluation (Jin and Zhang, 1993).

CONCLUSIONS

The minimum random sampling square number for straw and grain yield estimates was 10 m^2 (one square = approximately 1 m^2) under the condition of 5% relative deviation in a plot for field experiments of wheat. Because straw and grain yield data from random sampling squares in field trials were prone to large variations, it is strongly recommended to design a large plot as possible and to harvest the whole plot for straw and grain yield. The relative variation in plant height was lower than that in yield in a population. The minimum random sampling plant number for the plant height estimate was 20 plants in a plot under the condition of 1.5% relative deviation. Variations in the representativeness of the random sampling square number for straw and grain yield estimates were large.

ACKNOWLEDGEMENTS

This research was supported by the Department of Energy Conservation and Technology Equipment of China's National Energy Administration (Science and Technology Department, No. [2012] 32) and funded by China Datang New Energy Co. Ltd. and Henan Tianguan Group Co., Ltd.

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