COMBINED NITROGEN AND PHOSPHORUS APPLICATION SYNERGISTICALLY REGULATE GRAIN YIELD AND PROTEIN CONTENT IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract. To provide technical support for better quality and to get high-yield of winter wheat in Shanxi Province, four winter wheat genotypes are investigated in the field experiments to reveal the synergistic change regularity of grain protein content and yield, determine the protein threshold of winter wheat, and obtain the optimum fertilization application of nitrogen (N) and phosphorus (P). The study depicts P has a significant effect on the protein content and yield of winter wheat. Increasing the application rate of P can significantly increase the yield, while its influence on protein content varies within different varieties. Similarly, application rate of N has a prominent effect on both yield and protein content as both of which are enhanced with the increase of N application. We also record a synergistic increase in yield and protein content between certain intervals; the yield and protein content increase first, and then decrease with increasing applications of N fertilizer, which can be characterized with a parabola. Applying the appropriate amount of N and P can cause a synergistic increase for protein content and yield, and the most ideal threshold intervals of protein corresponding to the optimal P and N application rate are 150 kg/ha-200 kg/ha and 180 kg/ha-255 kg/ha, respectively.
Keywords: *threshold intervals of protein, nitrogen fertilizer, phosphorus fertilizer, genotypes of winter wheat, grain protein, grain yield*

Introduction

Winter wheat (*Triticum aestivum* L.) is an important grain crop in China and also the second top grain crop in Shanxi Province. However, medium and low-yield winter wheat with 3721.15 kg/hm^2 which is lower than the average yields in China with 5495.8 kg/hm^2 accounts for more than 50% of total wheat fields in Shanxi Province. Increasing the yield per unit area could improve self-sufficiency rate of wheat in Shanxi Province (Yang et al., 2010). Meanwhile, with the economic development and the improvement of people's living standard, purchasing the production of high yield, high quality and high efficiency has become an important goal of wheat production nowadays. Fertilizer application is one of the important factors that influence output and quality of wheat and an appropriate application amount of fertilizer to enhance the output and yield of wheat simultaneously is an important way to enhance production of wheat.

Both water and fertilizer conditions have a great effect on wheat yield and quality (Huang et al., 2015; Mostafa et al., 2018; Wang et al., 2019), with N fertilizer as the largest contributing factor (Bouacha and Nouaigui, 2014; Nan et al., 2015; Zhen et al., 2016). It is generally believed that within certain range of N application, the yield of wheat grain will be improved with the increasing application rates. But when N application amount exceeds a certain level, it can be non-significant and even decrease yield. Likewise, the grain protein content of wheat also shows a similar trend with the increasing application amount of N (Fan et al., 2021; Wang et al., 2021; Zhang et al., 2021). Appropriately increasing N application level can improve wheat yield and grain

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protein content, which can synchronously increase grain yield and protein content. Too high N were negatively correlated with grain protein and yield (Wang et al., 2003; Hawkesford, 2014; Zörb et al., 2018). Researchers also demonstrated similar findings indicating a "threshold value of protein content" (Lin et al., 2004). The effect of increasing yield reaches its maximum value when the N application amount remains 284 kg/hm^2 and the effect of increasing grain protein is the most significant when it is 384 kg/hm^2 (Deng and Jiao, 2021). Similarly grain yield of wheat and protein content increased with the increasing application amount of N at 270 kg/hm^2 but when N application amount is raised to 360 kg/hm^2, grain yield and protein content reduced (Xu et al., 2012).

Same is the case for P fertilizers which play a good role in promoting tiller and root growth of wheat, but its effect on grain yield and protein content remains ambiguous. Most of researches believed that the yield increased differentially in response to P fertilizer (Xing et al., 2015; Bekalu and Mano, 2016; Abbas et al., 2016). For example, Yu et al. (2013) reported that the yield of winter wheat had maximum values when P application amount was 172.5 kg/hm^2, but Xing et al. (2015) reported 240 kg/hm^2 P-fertilizer as optimum dose while wheat yield decreased at 480 kg/hm^2. Similar reports have been reported earlier by many researchers (Sun et al., 2006; Wang et al., 2006, 2009; Xing et al., 2015; Abbas et al., 2016; El-Sobky, 2017; Dambeniece-Migliniece et al., 2018).

It is of great importance to study the rational application amount of N and P to promote wheat production. Both N and P fertilizer application amount exhibit a strong relation to the variation of grain yield and protein content (Zhao and Yu, 2006; Zhao et al., 2010; Xu et al., 2012; Xing et al., 2015). Most interestingly, within this range there is a possibility that the grain yield and protein content may synergistically change (Bouacha and Nouaigui, 2014; Wei et al., 2021). Meanwhile, there are protein content threshold intervals with relatively high protein content and wheat yield (Lin et al., 2004). Previous studies have attached importance on the effects of N and P fertilizer on wheat yield and protein content, but there have been relatively few studies which focus on the effect of NP treatment on the relation and variation regularity between wheat yield and protein contents. The determination of protein threshold intervals is of therefore greater significance and could be important for production of high quality wheat. This study investigated the synergistic effects of N-P application rates on change regularity of winter wheat yield and the grain protein contents.

Materials and methods

Experiment details

Experiment was conducted at farming station of Shanxi Agricultural University in Taigu County, China from October 2013 to June 2015 with four varieties viz. V1, Jintai182; V2, Jintai170; V3, Jingdong12; V4, Lumai14.

Field experiment

The soil texture of experimental field at farming station is Calcaric Cambisols which developed from the loess parent material and has medium-level soil fertility. The content of organic matter, total nitrogen, available nitrogen, total phosphorus, available phosphorus and available potassium were 21.7 g/kg, 0.184%, 54.7 mg/kg, 0.12%, 17.5 mg/kg and 239.8 g/kg, respectively. Four different genotypes of winter wheat cultivars which were

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extensively used and strongly suggested by the local government were selected to conduct fertilizer experiment and the randomized block design were adopted for three repetitions. The details of grain quality for all winter wheat varieties were showed in *Table 1*.

Table 1. *The grain quality details of four selected winter wheat*

Varieties	Protein content (%)	Wet gluten (%)	Stable time (min)	Sedimentation value (ml)	Absorption (%)
Jintai 182 (V1)	16.17	32.8	3.6	29.5	59.2
Jintai 170 (V2)	16.49	35.1	21.2	60.6	61.8
Jingdong 12 (V3)	17.40/18.20	41.00/37.90	4.20/3.40	33.5/30.9	61.10/62.80
Lumai 14 (V4)	11.76	25.38	-	-	-

Each cultivar was treated by 3 P fertilizer levels and 4 N fertilizer levels (*Table 2*). With the addition of a control group P0N0, there were 13 treatments in total. The area of each block was 16 m² and there were guarding row among each block. Meanwhile, the row distance and plant density are 20 cm and 4.5×106 plant·hm^{-2}, respectively. The varieties of nitrogen and phosphorus fertilizers used in the experiment were urea and calcium superphosphate, and the base fertilizer was applied before sowing. The P fertilizer was applied at one time, N fertilizer used method of topdressing at the returning green stage, and the ratio of basal fertilizer and top-dressing was 1:1.

Table 2. *Combined application of N and P under different experiments of wheat varieties*

Treatments No.	Treatments	Amount of fertilizer application (kg/hm^2)		N/P
		N	P_2O_5	
1	N0P0	0	0	-
2	N1P1	105	100	1.05:1
3	N1P2	105	150	1:1
4	N1P3	105	200	0.525:1
5	N2P1	180	100	1.8:1
6	N2P2	180	150	1.2:1
7	N2P3	180	200	0.9:1
8	N3P1	255	100	2.55:1
9	N3P2	255	150	1.7:1
10	N3P3	255	200	1.275:1
11	N4P1	330	100	3.3:1
12	N4P2	330	150	2.2:1
13	N4P3	330	200	1.65:1

Determination of yield traits and grain protein contents

The yield attributes of plants were recorded from 5 plants per treatments and the proteins contents were quantified via Kjeldahl method.

Data analysis and processing method

The range transformation was used to standardize the values of wheat yield and the content of grain protein (eliminating dimension of quantity).

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$$y_i' = \frac{y_i - y_{min}}{y_{max} - y_{min}} \quad \text{(Eq.1)}$$

where, y_i' refers to yield (protein content) standard value, $y_i$refers to actually measured yield (protein content), y_{min} refers to the measured minimum output (protein content) and y_{max} refers to the maximum output (protein content). The figures were made by using the DPS, Excel 2013 and Origin softwares.

Results

Effect of different ratios of N and P on the yield of winter wheat

The effects of different combinations of *N and P applications* on the grain yields was analyzed (*Table 3*), and it showed that the grain yield of the four varieties were 4978.4 kg/hm², 5011.6 kg/hm², 5143.3 kg/hm² and 5146.2 kg/hm², respectively, under the condition of P0N0 in the control group. The yield of each variety had a trend of increasing initially and then decreasing with different nitrogen application ratio under the constant phosphorus application rate of each variety, and the variation range existed. For V1, there are significant differences under P1N2, P2N3, and P3N4 conditions; V2 under P1N3, P2N3, P3N2, P3N3, and P3N4 conditions, respectively; and V3 under P1N2, P1N3, P2N3, P2N4, and P3N3 conditions, respectively. There were significant differences in V4 under the conditions of P1N2, P1N3, P1N4, P2N2, P2N3, P3N2, and P3N3, respectively. Moreover, under different nitrogen fertilization treatments, the yield of each cultivar increased with the increase of phosphorus application, V1 was significantly different under N1, N3 and N4 conditions, and P3N1, P2N3, P3N3, P2N4 and P3N4 conditions; V2 wheat yield increased with phosphorus application, and increased with the increasing of the amount, and the difference was significant under N2, N3 and N4 conditions. The wheat yield of cultivar V3 increased with the increasing of phosphorus application under N1 and N4 conditions, and the difference was significant under P2N1, P3N1 and P3N4 conditions. Variety V4 increased significantly with the increase of phosphorus application under N2 and N3 conditions.

Table 3. *The effects of different N and P applications on grain yield of four varieties*

Variety	Treatment	N1	N2	N3	N4
V1	P1	5614.9±22.77 de	7174.7±25.25 c	6152.0±27.12 d	5156.3±21.21 e
	P2	5650.0±23.11 de	5927.6±22.67 d	6871.6±21.89 c	5890.4±24.23 d
	P3	7198.3±25.71 c	7335.4±24.98 bc	7342.4±24.37 bc	6071.3±22.91 d
V2	P1	5697.2±23.68 de	6160.7±24.25 d	7329.2±25.14 bc	5566.7±22.43 de
	P2	6368.1±22.97 cd	7415.7±25.09 bc	8840.0±29.05 a	6549.2±24.12 cd
	P3	6656.5±23.33 cd	7823.8±24.73 b	8913.8±26.35 a	7824.0±25.14 b
V3	P1	5268.7±22.26 e	6767.7±23.27 c	7867.4±25.11 b	5538.1±23.26 de
	P2	6099.4±24.15 d	7890.7±27.24 b	7120.8±28.16 c	6193.4±24.74 d
	P3	6657.4±23.78 cd	7322.5±29.03 bc	8372.6±28.33 b	6975.7±22.17 c
V4	P1	6102.4±23.36 d	7437.3±25.27 bc	8088.1±29.22 b	7880.0±27.81 b
	P2	6732.3±22.29 cd	9701.7±29.54 a	8848.5±28.25 a	6044.5±22.33 d
	P3	8261.9±26.74 b	9656.7±28.31 a	8925.3±27.71 a	7901.5±26.09 b

To clearly show the effect of different applications of N, P and its interactive combinations on the grain yield of four varieties, the radar maps (*Fig. 1*) which could

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represent the optimal combinations of N and P application for achieving the highest grain yields and also visually indicate the interval distribution effect between the different N levels, and different P levels.

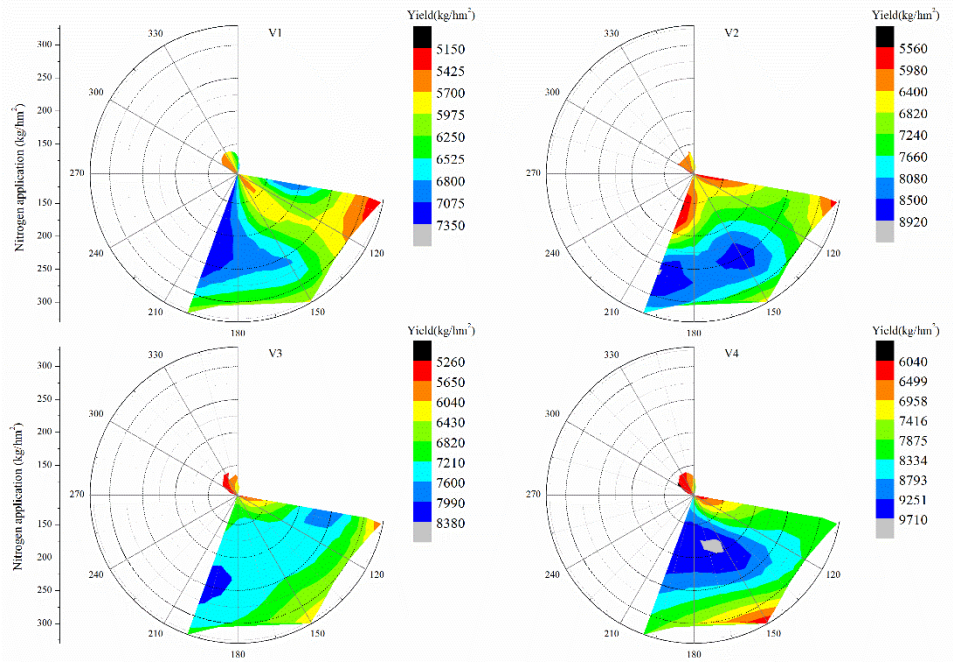

Figure 1. *The grain yield contour map of wheat varieties with different ration of N and P*

It shows that the relation of different wheat cultivars towards different N and P fertilizer doses is presented. When P application amount remained constant, the yield of winter wheat showed a trend of increasing firstly and then decreasing with the increasing N application. When N application amount remained constant, the yield of winter wheat increased with the increasing P application. Under the condition of low and high P application, V1, V3 and V4 had different degrees of insensitivity to N fertilizer respectively. Especially, when P application was 200kg/ hm², V1 exhibited the highest insensitivity towards N fertilizer and acquired higher yield when N application amount kept within the range of 0-275 kg/hm^2. The cultivars V1 and V3 exhibited higher yield under low and high P application, while V2 and V4 showed remarkably increasing trends with the increased P application. Above all, the yield of four winter wheat cultivars increased first and then decreased with the increased N application, and almost all reached the highest levels at N2 or N3 treatments respectively. By contrast, too low or high N applications resulted in lower yield of different cultivars in response to different P treatments.

Effect of different N and P application rates on protein content of four varieties

The effects of different combinations of N and P applications on the grain yields was further analyzed (*Table 4*), and it showed that under the condition of P0N0 in the control group, the protein contents of the four varieties were 8.61%, 7.54%, 8.93% and 6.07%, respectively. There were significant differentially-expressed in the highest protein content under different nitrogen and phosphorus combined application ratios. Under low P treatment, V1 and V2, V4 had significant differences in the highest protein content, and

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V3 had significant differences with V2, V4 in the highest protein content; under medium P treatment, V2 and V1 and V3 had significant differences in protein content, and V4 had significant differences with V1 and V3. Under high P treatment, V1 had significant differences with V2 and V4 protein content, and V3 had significant difference with V2 and V4 protein content in their highest values. Overall, under the condition of a certain amount of phosphorus fertilizer, the four varieties of winter wheat showed higher protein content under the medium nitrogen treatment, and most of the treatments were significantly different.

Table 4. *The effects of different N and P applications on protein content of four varieties*

Variety	Treatment	N1	N2	N3	N4
V1	P1	9.42±0.37 d	13.26±0.51 b	16.69±0.39 a	10.80±0.42 cd
	P2	9.52±0.43 d	11.22±0.28 c	13.39±0.25 b	10.13±0.53 cd
	P3	9.77±0.26 d	14.27±0.32 ab	13.02±0.35 b	11.84±0.44 c
V2	P1	8.48±0.22 de	13.22±0.18 b	15.48±0.24 cd	9.10±0.35 d
	P2	8.67±0.16 de	10.86±0.21 cd	15.27±0.37 cd	10.92±0.19 c
	P3	10.06±0.27 cd	13.48±0.31 b	15.26±0.36 c	10.46±0.47 cd
V3	P1	9.11±0.21 d	11.11±0.29 c	15.96±0.33 a	12.97±0.41 b
	P2	9.43±0.29 d	12.93±0.36 b	17.22±0.43 a	9.89±0.37 d
	P3	10.21±0.25 cd	13.20±0.29 b	17.87±0.32 a	13.75±0.21 b
V4	P1	10.76±0.49 cd	11.16±0.14 c	12.56±0.58 bc	9.76±0.28 d
	P2	6.42±0.11 e	8.62±0.19 de	10.97±0.21 c	6.82±0.15 e
	P3	8.31±0.16 de	9.00±0.22 d	7.86±0.34 e	6.24±0.18 e

The radar maps (*Fig. 2*) was also made to clearly showed the optimal combinations of N and P application for achieving the highest protein content and also visually indicate the interval distribution effect between the different N levels, and different P levels.

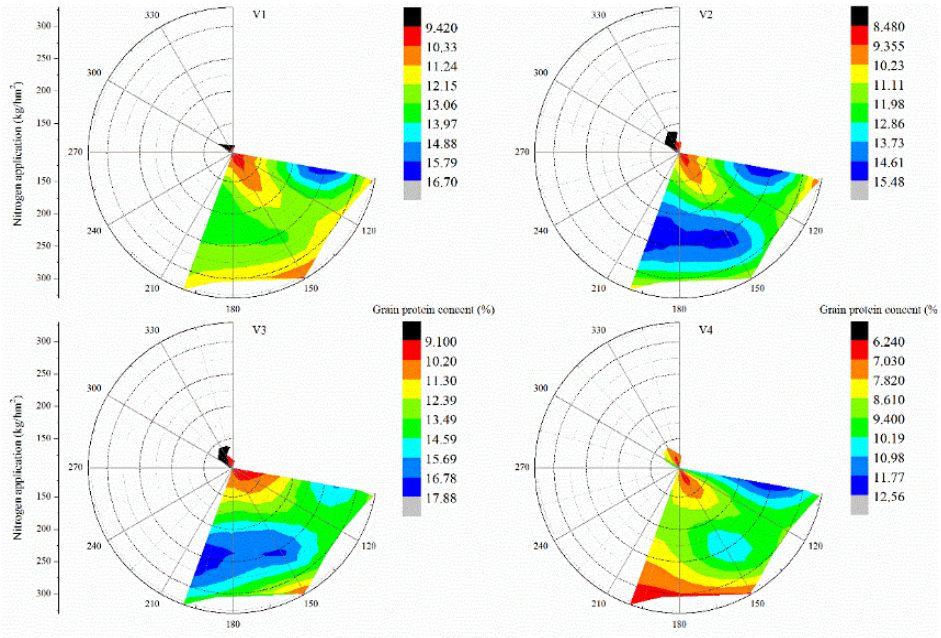

Figure 2. *The grain protein contour map of wheat varieties with different ration of N and P*

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The *Fig. 2* indicates that the grain protein content of four winter wheat cultivars exhibited differential trends of variation with the increase of P application. The cultivar V1 exhibited decrease initially and then slowly increased with the increasing application of P and reached peak values when treated with low P treatment. The grain protein content of V2 and V3 exhibited the same trends as that of V1 with the increasing application of P, and reduced to the lowest value within the range of low to medium P application. The grain protein content of V4 reached the highest level when given high P application, and then gradually decreased with the increasing application of P. Overall, the grain protein content of V1 and V4 reached the highest levels when given lower P treatment, while the grain protein content of V2 and V3 increased highest levels when given medium and high P treatment.

When P fertilizer amount remained constant, the grain protein content of four winter wheat cultivars increased initially, and decreased with the increasing application of N reaching peak values in the range of N2 to N3. Under the condition of low P treatment, peak values for grain protein content of all four cultivars were evident in N3 treatment; under the condition of medium P treatment, higher values of four cultivars when given N3 treatment, of which V2, V3 exhibited higher peak values. The cultivar V4 and V1 had least values under the condition of high P treatment, grain protein content of V1, V4 were highest (remaining relatively low) when given N2 treatment. While, the grain protein content of V2 and V3 reached to the highest values (remaining relatively high) under the N3 treatment.

Threshold intervals of grain yield and protein

To find out synergistic increase intervals of yield and protein content and the corresponding N application amount under different P fertilizer treatments, the research adopted Range Transformation to evaluate standard value, and standard value variation curve of yield and protein were drawn according to the variation of N application amount in *Fig. 3*.

The *Fig. 3* indicated that V1 and V4 showed poor synergistic effects under low P application. However, the V2 showed the optimum synergistic effect in terms of protein content and yield when the P application amount was within the range of medium and high levels and the N application amount was close to N3. The V3 exhibited a mediate synergistic effect when low or high P fertilizer was applied.

All figures *(Figs. 1 to 3) also* indicated that the standardized yield and protein content of the four cultivars can be shown with convex parabolas. With the increase of fertilizer application, the yield and protein content of each cultivar simultaneously increase to the maximum intervals with the increase of fertilizer application, which is the optimum intervals for high yield and high quality, also called the protein threshold intervals.

For V1, the protein threshold interval was (14.8% and 16.6%) under low P treatment and the corresponding optimal amount of N application was 217.5-255 kg/hm^2; its protein threshold interval was (11.6% and 13.39%) under medium P treatment and the corresponding optimal amount of N application was 217.5-292.5 kg/hm^2; the protein interval was (13.02% and 15.48%) under high P treatment and the corresponding optimal amount of N application was 180-255 kg/hm^2. For V2, the protein threshold interval was (12.29% and 14.35%) under low P treatment and the N application amount was 217.5-292.5 kg/hm^2; the protein threshold interval was (13.07% and 15.27%) under medium P treatment and the N application amount was 217.5-292.5 kg/hm^2; the protein threshold interval was (12.77% and 15.26%) under high P treatment, and the N

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application amount was 217.5-292.5 kg/hm^2. For V3, the protein threshold interval under low P treatment was (13.54% and 17.96%) and the corresponding optimal amount of N application was 217.5-292.5 kg/hm^2; the protein threshold interval under medium P treatment was (12.93% and 17.22%) and the N application amount was 180-255 kg/hm^2; the protein threshold interval under high P treatment was (15.54% and 17.87%) and the N application amount was 217.5-292.5 kg/hm^2. For V4, the protein threshold interval under low P treatment was (11.86% and 12.56%) and the N application amount was 217.5-255 kg/hm^2; the protein threshold interval was (8.90% and 10.97%) under medium P treatment, the N application amount was 217.5-255 kg/hm^2; the protein threshold interval under high P treatment was (8.43% and 9.00%), and the N application amount was 142.5-217.5 kg/hm^2.

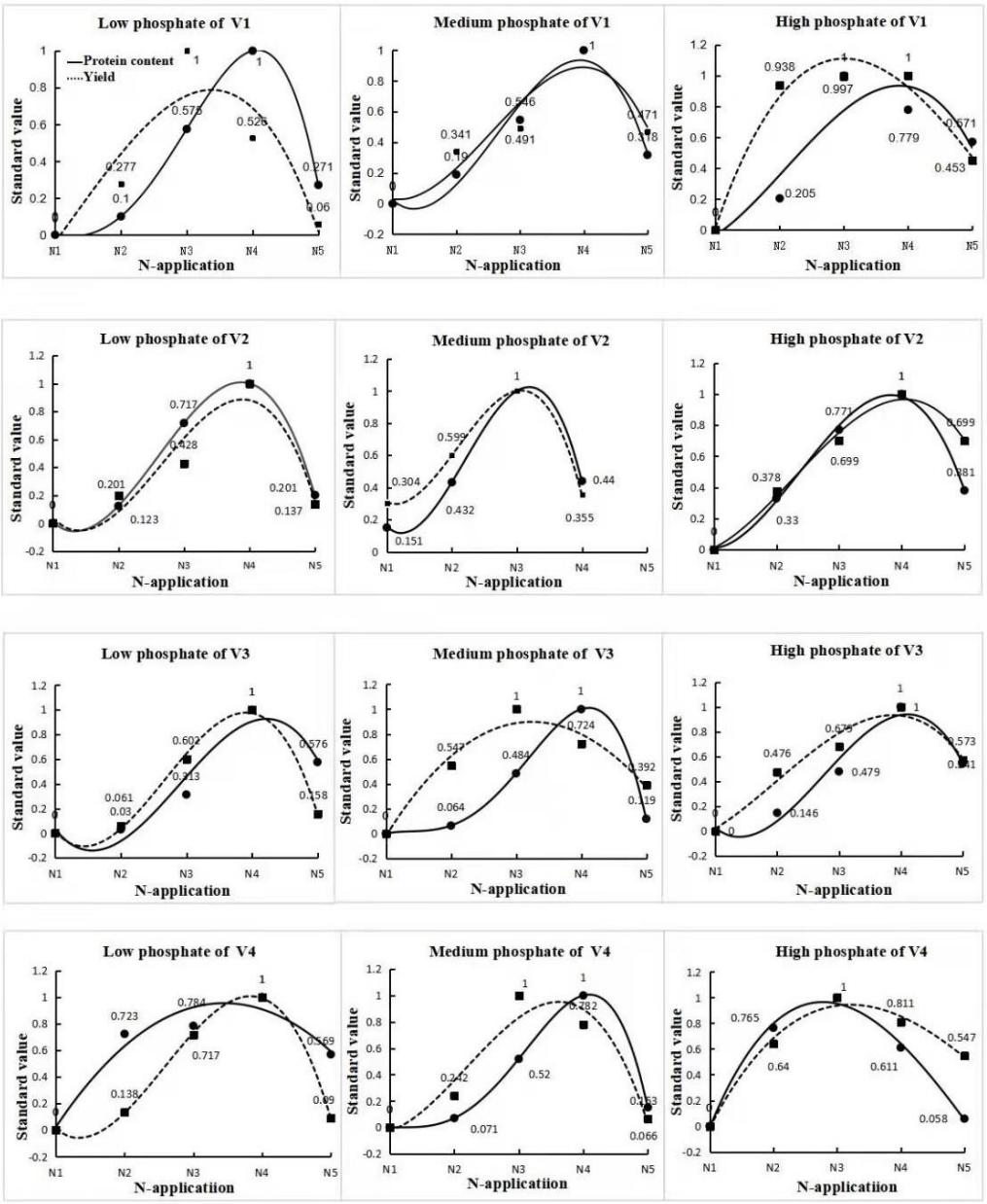

Figure 3. *Dynamics of yield and protein content in different P-application of the 4 varieties*

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The fitted equation of the grain protein content and yield of winter wheat

Under the condition of applying a certain amount of P fertilizer, a polynomial regression was employed to establish the fitted equation of grain protein content and yield of winter wheat. The cubic equation with one variable can be used to demonstrate the changing regularity of protein content and yield with the increase of N fertilizer application (*Fig. 3*). As *Table 5* shows, the R^2 of the model after fitting is above 0.82 and the regression effects of the equations were good.

Table 5. *Fitted equation of N utilization efficiency on yield and protein under different P-application*

Varieties	Treatments	Fitted equation $y=a_0+a_1x+a_2x^2+a_3x^3$				
		a_0	a_1	a_2	a_3	R^2
V1	P1	-0.4664	0.3254	0.1369	-0.0365	0.8252
		1.2432^p	-2.1308^p	1.0248^p	-0.1274^p	0.9900
	P2	0.4756	-0.9138	0.5366	-0.0706	0.8955
		0.9768^p	-1.7106^p	0.8589^p	-0.1085^p	0.9762
	P3	-1.5864	2.0407	-0.4629	0.0274	0.9684
		-0.1170^p	-0.1737^p	0.3012^p	-0.0481^p	0.8518
V2	P1	1.1852	-2.0019	0.9684	-0.1218	0.8983
		1.1212^p	-2.0028^p	1.0108^p	-0.1294^p	1
	P2	0.5856	-1.1308	0.6498	-0.0864	0.9589
		1.0796^p	-1.8147^p	0.8624^p	-0.1048^p	0.9396
	P3	0.0232	-0.2793	0.3103	-0.0454	0.9905
		0.3554^p	-0.8385^p	0.5685^p	-0.0799^p	0.9977
V3	P1	1.4212	-2.4219	1.1508	0.1433	0.9959
		1.4048^p	-2.2543^p	0.9870^p	-0.1137^p	0.9429
	P2	-1.0524	1.2367	-0.2061	0.0032	0.9563
		1.5384^p	-2.5613^p	1.1866^p	0.1461^p	0.9688
	P3	-0.1344	-0.0437	0.2357	-0.0396	0.9572
		0.9752^p	-1.6635^p	0.8023^p	-0.0972^p	0.9677
V4	P1	1.1680	-2.0842	1.0546	-0.1362	0.9995
		-0.9068^p	1.0937^p	-0.1650^p	0.0012^p	0.9342
	P2	0.1900	-0.6876	0.5539	-0.0845	0.9308
		1.4628^p	-2.4561^p	1.1498^p	-0.1421^p	0.9789
	P3	-1.2454	1.5398	-0.3221	0.0171	0.9861
		-1.6442^p	2.1131^p	-0.5074^p	0.0305^p	0.9944

R^2 refers to determination coefficient. a_0, a_1, a_2, a_3 refer to the coefficients of yield equation. a_0^p, a_1^p, a_2^p, a_3^p refer to the coefficients of protein content equation

Discussion

Through fertilization experiments on four cultivars of winter wheat, our study verified that protein content and yield can change synergistically under certain fertilization condition, and there are protein threshold intervals, within which the grain protein content and yield can reach up to higher levels. Under the same conditions of ecological circumstance, soil fertility, and fertilizer application, the maximum protein content of each cultivar is different, which may be related to the differences in the nitrogen assimilation ability, nutrient redistribution ability, as well as genotypic differences of

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plants' total nitrogen content at flowering stage and nitrogen transferring (Sun et al., 2006; Wang et al., 2006, 2009).

A previous study suggested that the yield of wheat decreases with the increase of P application after it has reached a certain amount, showing a trend of increasing firstly then decreasing with the increase of P application (Xing et al., 2015; Abbas et al., 2016; Zhang et al., 2018). Our experiment indicated that the yield of four cultivars under high P application > the yield under medium P application > the yield under low P application. In contrast to our research findings, Yu et al. (2013) reported that wheat yield reaches to the maximum level when the applied amount of P fertilizer is 72.5 kg/hm^2, which is because the effective phosphorus content of the soil is 38 mg/kg. Xing identified that the yield of winter wheat will reach to the highest level when P application amount is 240 kg/hm^2 and that the yield will decrease when P application amount is 400 kg/hm^2. The effective phosphorus content of the soil in Xing's experiment is 18.59 mg/kg (Xing et al., 2015), which is basically the same as that of our experiment. In our experiment, the total phosphorus content of the soil accounts for 0.12%, and the effective phosphorus content is 17.58 mg/kg, suggesting the phosphorus content of the soil is at the medium level. The optimal amount of P application in Xing's study is basically consistent with the results of our experiment. Therefore, the inconsistency of various studies in the optimal amounts of P application may be result from the differences of soil fertility.

Under the condition that N application amount is certain, the P fertilizer had different influences on the protein content of the four cultivars. The V3 had higher protein content under medium or high P application than low P application, which is probably because the nitrogen use efficiency is greatly improved under the treatment of medium or high P fertilizer (El-Sobky, 2017). The V4 had higher protein content under the treatment of low P fertilizer than medium or high P fertilizer, which is consistent with the published research results (Fu et al., 2008; Wang et al., 2009). It is important to mention that the quality of medium and weak gluten wheat decreases with the increase of P fertilizer. In our experiment, the protein content of V2 initially decreased and then increases with the increase of P fertilizer application and minimum at under medium and low P application. At the same time, according to the change in the yield of this cultivar, a conclusion can be drawn that the application of medium and high P fertilizer ensures both high yield and high protein content. Therefore, in practice, we should take into consideration that wheat varieties have influences on the effects of fertilization.

The changing regularities of protein content and yield with the increase of P fertilizer application can be shown with convex parabolas. N application amounts corresponding to the maximum yield and protein content were not exactly the same. Under some treatments, the highest critical values of yield and protein content corresponded to the same N application amount. Under some treatments, the protein content reaches to the critical values earlier than the yield with the increase of N fertilizer. While under other treatments, the yield reached to the critical values earlier than protein content when N application increases. But in the three cases, when the yield and protein content reach the critical values, the corresponding N application amount ranges from N2 to N3, which is probably because excessive application of N fertilizer reduces net photosynthetic rate of wheat. When nitrogen use efficiency decreases, the flat grains will increase and the yield will decrease. When the critical values of yield and protein content correspond to the same N application amount, the yield and the protein content were always positively correlated to each other with the increase of N application. However, when the two critical values corresponded to different N application amount, the yield had a negative correlation with

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protein content within a certain extent. Therefore, some previous studies have concluded that yield and protein content are negatively correlated (Wang et al., 2003), while other studies have suggested that yield and protein content can increase simultaneously under suitable conditions (Cooper et al., 2001; Danid and Triboy, 2002), which is because N application amounts corresponding to the critical values of yield and protein content are diverse under different experimental conditions. At the same time, the study has figured out the synergistic intervals of yield and protein content with the increase of N application under different amounts P application, according to fitted equation of N utilization efficiency on yield and protein under different P application (*Table 5*).

Conclusion

In this study, four winter wheat cultivars were treated with combined N and P fertilizers to study the synergistic change regulation of grain protein content and yield for the purpose of obtaining the protein threshold intervals of winter wheat. The results clearly showed that grain yield and protein content could synergistically increase under the medium and high P fertilizer treatment and under the condition of N fertilizer treatment within the range from N2 to N3. At the same time, the protein threshold intervals can be calculated by the fitted equation, however, the difference among the winter wheat genotypes need to be further explored as their varied genetic characteristics which will ultimately affect the yield components and grain quality even though at the same fertilizer treatments. Moreover, the combined effect between the water and nitrogen, phosphorous need also revealed as the absorption of nitrogen, phosphorous by crops is affected by the soil water which was hard to be controlled in our study. Thus, finding out the interactive effect among these factors would be beneficial for understanding the relationship between the grain yield and the protein content.

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