



Assess Effect of Different level of Nitrogen Fertilizer on Redistribution, Efficiency and Contribution of Redistribution and Current Photosynthesis of Wheat Genotypes

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ABSTRACT

BACKGROUND: Management of nutrients, especially nitrogen, in order to wheat economic production and maintain sustainable agriculture to provide food security, is considered to have an important priority. Fertilizer management plays an important role for obtaining satisfactory yields and increase crop productivity.

OBJECTIVES: This research was done to evaluate response of crop production and agrophysiological traits of wheat genotypes to apply different level of nitrogen fertilizer.

METHODS: Current study was conducted according split plots experiment based on randomized complete block design with three replications. Main factor included four levels of nitrogen fertilizer ($N_1=60$, $N_2=120$, $N_3=180$, $N_4=240$ kg.ha⁻¹) and sub plots consisted four genotypes of Bread (V_1 : S85-19 as line, V_2 : Chamran) and Durum (V_3 : Behrang, V_4 : Dena) Wheat.

RESULT: According result of analysis of variance effect of different level of nitrogen fertilizer, genotypes and interaction effect of treatments on all studied traits was significant at 5% probability level. Evaluation mean comparison result of interaction effect of treatments on all measured traits revealed the highest amount of seed yield (610 gr.m⁻²), number of spike per square meter (1010), rate of redistribution (162.97 gr.m⁻²), efficiency of redistribution (0.24 gr.gr⁻¹), contribution of redistribution (50.50%), rate of current photosynthesis (737.67 gr.m⁻²), efficiency of current photosynthesis (0.95 gr.gr⁻¹) and contribution of current photosynthesis (83.50%) were noted for 180 kg.ha⁻¹ nitrogen fertilizer and Chamran genotype and lowest amount of mentioned traits belonged to nonuse of fertilizer and S-85-19 line treatments.

CONCLUSION: Finally according result of current study application 180 kg.ha⁻¹ nitrogen fertilizer and Chamran genotype had the maximum amount of studied traits and it can be advice to producers in studied region.

KEYWORDS: *Durum, Nutrition, Physiology, Remobilization, Seed yield.*

1. BACKGROUND

Nitrogen (N) is essential for all biological process that occurs in the plant. A sub-optimal supply of N limits the expression of yield potentials of green bean varieties (Dauda *et al.*, 2015). Nitrogen deficiency is frequently a major limiting factor for high yielding crops all over the world (Salvagiotti *et al.*, 2008). The growth and yield of a crop can be adversely affected by deficient or excessive supply of any one of the essential nutrients. However, in intensive agriculture nitrogen is the major nutrient which determining crop yield. Nitrogen as an essential constituent of cell components having direct effect on growth, yield and quality of crop. Plant growth is affected more due to deficiency of nitrogen than that of any other nutrient. Nitrogen fertilization influences dry matter yield by influencing leaf area index, leaf area duration and photosynthetic efficiency (Mohan *et al.*, 2015). Nitrogen is known as an essential element from vegetative stage to physiological maturity (Ali, 2011) and one of the main inputs of wheat agriculture with expecting optimum yield. Nowadays, the use of nitrogen fertilizers is increasing continuously. According to the research conducted by Good and Beatty (2011), the total nitrogen application estimated 5.8 million tons in 1987 and will rise to approximately 6.151 million tons in 2050. Availability of nitrogen is important for growing plants. It is a main constituent of protein and nucleic acid molecules. It is also a part of chlorophyll molecules. It is well known that the use of fertilizer helps in production and is a quick method re-

sulted in the best yields (Farooqui *et al.*, 2009). The most important role of N in the plant is its presence in the structure of protein and nucleic acids which are the most important building and information substances of every cell. In addition, N is also found in chlorophyll that enables the plant to transfer energy from sunlight by photosynthesis. Thus, the supply of N to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Consequently, it influences cell size, leaf area and photosynthetic activity (Walley *et al.*, 2005). Therefore, adequate supply of N is necessary to achieve high yield potential in crops. In general, N deficiency causes a reduction in growth rate, general chlorosis, often accompanied by early senescence of older leaves, and reduced yield (Erman *et al.*, 2011). McKenzie and Hill (1995) studied the effects of two levels of N applications (0 and 50 kg N ha⁻¹) on chickpea and reported that the increase of N rate from 0 to 50 kg N ha⁻¹ significantly enhanced seed and dry matter yield, harvest index, number of pods per plant and 1000 seed weight. Nitrogen deficiency generally results in stunted growth and chlorotic leaves caused by poor assimilate formation that leads to premature flowering and shortening of the growth cycle (Lincoln and Edvardo, 2006). Researchers reported that the increase in nitrogen consumption increases the number of spikes per unit area, which can increase vegetative growth and, consequently, increase the amount of tillering due to nitrogen consumption. In such a situation, the number of

fertilized tillers per unit area increases and the number of spikes per unit area also increases (Mosanaei *et al.*, 2017). According to the research of Mosanaei *et al.* (2017), the effect of nitrogen fertilizer on the number of wheat spikes was significant, which was consistent with the results of the present study. Nitrogen increases the biomass production and increases the possibility of retransmission of photosynthetic materials, producing more seeds per spike and better filling them after flowering, which will increase seed yield (Shanggan *et al.*, 2000). The variability of the harvest index in the plants depends on the difference in the production of the assimilates during the seed filling and retransplantation of the assimilates before the pollination of each genotype and the strength of the reservoir (Nourmohammadi *et al.*, 2001). Increase of harvest index due to the increase of nitrogen fertilizer in maize can physiologically attribute to the increase of leaf area continuity and, nitrogen availability. In fact by creating balance between the nutrients bio-fertilizers increase both vegetative and reproductive growth and by creating adequate destination (seed), the assimilates will mobilize into seeds and ultimately the harvest index of plant seed increase (Araei *et al.*, 2014). The most effective environmental factor on wheat quality is nitrogen fertilization. At the same time, the degree of influence is affected by annual weather conditions and by residual soil nitrogen (López-Bellido *et al.*, 2001). Therefore, proper management of N fertilizer is essential to ensure high quality wheat production. Design of fertilizer applica-

tion regimes should combine rate, timing, splitting, and source of application, with a view to optimizing wheat yield and its quality (Abedi *et al.*, 2010). Giambalwo *et al.* (2010) in a study on durum wheat genotypes at low nitrogen amount declared that nitrogen use efficiency had no considerable significant difference among genotypes. There are some evidence demonstrating correlation between available nitrogen and accumulation biomass, but it is so tough to quantify that. A comparing experiment was carried out on durum wheat, bread wheat and barley crop during years 2004/2005/2006/2007. The result showed that nitrogen uptake correlated with grain yield and final biomass at maturity advent (Cossani *et al.*, 2012). Wang *et al.* (1997) showed that source reduction caused a decrease in the allocation of dry matter to the sheath and stem, and promoted the reserve photosynthetic to be reallocated to grain. The effect of sink reduction was contrary, indicating that grain sink size was not a factor limiting the production of photosynthesis, but controlled the partitioning of photosynthesis.

2. OBJECTIVES

This research was conducted to evaluate response of seed yield and agro-physiological traits of wheat genotypes to use different level of nitrogen.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

Current study was carried out to assess the effect of different levels of nitrogen fertilizer on seed yield and physiological indexes of four wheat geno-

types along 2010-2011 in Ahvaz city in south west of Iran. Experimental site located at altitude 31° 36' N and longitude 48° 53' E and 51 m above the sea level. Statistical pattern was split plots experiment according randomized complete block design with three replications. Main factor included four levels of nitrogen fertilizer ($N_1=60$, $N_2=120$, $N_3=180$, $N_4=240$ kg.ha⁻¹, Urea Source)

and sub plots consisted four genotypes of Bread (V_1 : S85-19 as line, V_2 : Chamran) and Durum (V_3 : Behrang, V_4 : Dena) Wheat. Nitrogen fertilizer was consumed twice (a half during the greening and the rest during the stem elongation) equally. To determine soil properties of field, sampling test was done before sowing. Results of soil analysis were shown in table 1.

Table 1. Physical and chemical properties of studied field

Soil depth (cm)	Ec (ds.m ⁻¹)	pH	Absorbable elements (ppm)			Soil tissue components (%)			Soil type
			Total nitrogen	Phosphorus	Potassium	Clay	Silt	Sand	
0-20	2.7	8.1	740	16.1	210	38.5	44	17.5	Slit clay loam
20-40	2.4	8	380	7.2	120	44.3	42	13.7	

3.2. Farm Management

After ground preparation, the soil feeding process was carried out based on the results of soil sample analysis in the laboratory. Nitrogen fertilizer from urea source was consumed at 252 gram per plot. One-third deduction was applied before planting as basal, next section at end of tillering and final one-third deduction applied at the spike emergence stage. Phosphorus (P₂O₅) fertilizer was obtained from triple super phosphate source and was applied in amount 120 gram per plot at pre-planting stage. Potassium fertilizer was applied from the potassium sulfate source at 120 gr per plot. To combat and try control the broadleaf and narrow leaf weeds, Duplosan Super (2.5 L.ha⁻¹) and topic (1 L.ha⁻¹) herbicides was used at the end of tillage stage and before application of topdressing fertilizer, respectively.

3.3. Measured Traits

In order to determine the yield two planting lines from each plot harvested and after the removal of marginal effect were carried to the laboratory and were placed in the oven at 75°C for 48 hours and after ensuring that the samples were completely dry, they were weighted and finally the total yield was measured. In order to evaluate remobilization efficiency and contribution and current photosynthesis seven days after flowering from each plot after removing the effects of lateral margin, five plants were harvested and total dry weight was measured. At the end of plant growth grain yield and related traits were calculated using the relationship. Agro-physiological traits were calculated by the following equations (Papakosta and Gagianas, 1991):

Equ.1. $R = Y_2 - Y_1$

R= Redistribution of storage material
(gr.m^{-2})

Y_2 = Dry weight of vegetative organs at anthesis (gr.m^{-2})

Y_1 = Dry weight of vegetative organs at Maturity

Equ.2. Efficiency of redistribution
(gr.gr^{-1}) = R/Y_2

Equ.3. Contribution of redistribution
(%) = $R/\text{seed yield}$

Equ.4. Current photosynthesis (gr.m^{-2})
= seed yield-redistribution of storage material

Equ.5. Current photosynthesis efficiency (gr.gr^{-1}) = Rate of current photosynthesis/dry weight of vegetative organs at anthesis.

Equ.6. Contribution of current photosynthesis (%) = $100 - \text{contribution of redistribution (%)}$

3.4. Statistical Analysis

Data analysis of variance was done via using SAS software (Ver. 8) and the means were compared by Duncan's multi range test at 5% probability level.

4. RESULT AND DISCUSSION

4.1. Seed yield

According result of analysis of variance effect of nitrogen fertilizer, genotypes and interaction effect of treatments on seed yield was significant at 5% probability level (Table 2). Evaluation mean comparison result of interaction effect of treatments indicated maximum seed yield (610 gr.m^{-2}) was noted for 180 kg.ha^{-1} nitrogen and chamran genotype and lowest one (330 gr.m^{-2}) belonged to nonuse of nitrogen and S-85-19 line (Table 3). Saeedin (2016)

evaluated the correlation between biological yield and seed yield of cowpea and reported a positive and significant correlation between mentioned traits. Its seem biological yield increased because of accumulation of photosynthetic products (source products) and high potential of seeds (reservoir) for absorption and accumulation of dry matter. Therefore, any increases in seed yield also increases the biological yield. However, less dry matter is accumulated in case of micronutrient deficiency, which decreases the biological yield. In general, since early maturation genotypes begin earlier reproductive stages, they have more time for growth and filling the seed, whereas delayed genotypes have less time for filling and growth of the seed due to their delay in beginning reproductive stages and coincidence of developmental stages and seed growth with the heat of the end of season (Modhej and Lack, 2011). If the storage of photosynthetic materials is greater, the remobilization and volume of transferred material to the seed is higher, and more material will transferred to the seed at the same time. Any factor causing further transitivity to seed increases the seed filling rate and finally improves the seed yield (Yang *et al.*, 2000).

4.2. Number of spike per square meter

Result of analysis of variance revealed effect of nitrogen fertilizer, genotypes and interaction effect of treatments on number of spike per square meter was significant at 5% probability level (Table 2). Assessment mean comparison result of interaction

effect of treatments indicated maximum number of spike per square meter (1010) was noted for 180 kg.ha⁻¹ nitrogen and chamran genotype and lowest one (570) belonged to nonuse of nitrogen and S-85-19 line (Table 3). Some researchers reported the increase of nitrogen causes a significant increase of the number of tillers per plant and fertilized tillers, leaves surface and durabil-

ity of flag leaf, biological yield, number of spike per square meter and number of seeds per spike and the positive and significant effects of these traits on the seed yield, also a positive correlation between the number of seeds per spikelet and the number of spikelet per spike with the seed yield (Ehdaie and Waines, 2001; Kumar *et al.*, 2001).

Table 2. Result analysis of variance of measured traits

S.O.V	df	Seed yield	No. spike per m ²	Rate of redistribution	Efficiency of redistribution
Replication	2	173 ^{ns}	57.85 ^{ns}	72 ^{ns}	0.0000450 ^{ns}
Nitrogen (N)	4	44569*	3983*	13584*	0.1064442*
Error I	8	3516	291	140.8	0.0009117
Genotypes (G)	3	31876*	8178*	2975.1*	0.1031667*
N × G	12	92376*	1779*	1824.8*	0.1049542*
Error II	30	2218	101	120	0.0005383
CV (%)	-	12.23	10.71	9.85	14.9

^{ns, *} and ^{**}: no significant, significant at 5% and 1% of probability level, respectively.

Continue table 2.

S.O.V	df	Contribution of redistribution	Rate of current photosynthesis	Efficiency of current photosynthesis	Contribution of current photosynthesis
Replication	2	31.65 ^{ns}	170 ^{ns}	0.078 ^{ns}	30.66 ^{ns}
Nitrogen (N)	4	581.41*	38864*	4.934*	572.42*
Error I	8	30.88	1938	0.06	11.89
Genotypes (G)	3	392.52*	40246*	2.21*	363.57*
N × G	12	424.82*	93960*	3.45*	436.72*
Error II	30	23.44	1626	0.02	3.47
CV (%)	-	11.13	9.42	7.75	6.75

^{ns, *} and ^{**}: no significant, significant at 5% and 1% of probability level, respectively.

4.3. Rate of redistribution

According result of analysis of variance effect of nitrogen fertilizer, genotypes and interaction effect of treatments on rate of redistribution was sig-

nificant at 5% probability level (Table 2). Assessment mean comparison result of interaction effect of treatments indicated maximum rate of redistribution (162.97 gr.m⁻²) was noted for 180 kg.ha⁻¹

¹ nitrogen and chamran genotype and lowest one (53.50 gr.m⁻²) belonged to nonuse of nitrogen and S-85-19 line (Table 3). Schneider (1993) stated that two types of carbohydrate sources are providing photosynthetic material at grain filling period. Current photosynthetic products that are transferred directly to the grain and redistribute the photosynthetic materials stored in the stored tissues. Naseri *et al.* (2010) reported that the highest grain yield and biologic yield were obtained in 160 and 240 kg N.ha⁻¹ with 5100 and 14360 kg.ha⁻¹, respectively. Although in material movement process from the source to the sink the vascular system is not restricting, but due to the fact that in remobilization process in both parts of material accumulation in the vegetative parts while their movement and remobilization, energy will be consumed, therefore any increase in photosynthesis persistency and the accumulation of materials will be preferred to materials remobilization (Flood *et al.*, 1995). Yang *et al.* (2001) concluded that senescence induced by controlled soil drying during grain filling can promote the remobilization of presorted assimilates to the grains, accelerate grain filling, and improve yield in cases where senescence is unfavorably delayed by heavy use of nitrogen.

4.4. Efficiency of redistribution

Result of analysis of variance showed effect of nitrogen fertilizer, genotypes and interaction effect of treatments on efficiency of redistribution was significant at 5% probability level (Table 2). Evaluation mean com-

parison result of interaction effect of treatments indicated maximum efficiency of redistribution (0.24 gr.gr⁻¹) was noted for 180 kg.ha⁻¹ nitrogen and chamran genotype and lowest one (0.07 gr.gr⁻¹) belonged to nonuse of nitrogen and S-85-19 line (Table 3). Sources of photosynthetic material for grain filling include current photosynthesis, re-transfer of photosynthetic materials stored in vegetative organs before pollination and redistribution of stored materials in vegetative organs from pollination stage to beginning of linear growth grains (Naderi, 2001). Bahrani *et al.* (2011) demonstrated that grain-filling rate of wheat, while affected by water deficit, is maintained above what is expected from post-anthesis dry matter accumulation because remobilization of assimilates to the grain continued despite a reduction in C assimilation. Stems were more important than the other parts of the plant in the remobilization of dry matter to grain during the grain-filling period. The excessive use of N in Iran results in unfavorably delayed senescence. Early senescence induced by water stress could increase the rate of grain filling and improve kernel weight in this case. It was concluded that stored carbohydrate had provided an important buffer against water stress during grain filling, in terms of yield.

4.5. Contribution of redistribution

According result of analysis of variance effect of nitrogen fertilizer, genotypes and interaction effect of treatments on contribution of redistribution was significant at 5% probability level (Table 2).

Table 3. Interaction effect results of nitrogen fertilizer and genotypes on measured traits

Nitrogen Fertilizer (kg.ha ⁻¹)	Genotype	Seed yield (gr.m ⁻²)	No. spike per m ²	Rate of redistribution (gr.m ⁻²)	Efficiency of redistribution (gr.gr ⁻¹)
Control	Dena	340d	873b	72.41fg	0.08f
	Behrang	369d	833b	74.46fg	0.08f
	Chamran	423c	899b	78.57f	0.09ef
	S-85-19	330e	570d	53.50g	0.07g
60	Dena	400c	817b	90.30e	0.10e
	Behrang	410c	777bc	85.86e	0.11e
	Chamran	500b	893b	129.06d	0.12d
	S-85-19	433bc	673c	84.23e	0.09ef
120	Dena	450bc	917ab	125.67c	0.13cd
	Behrang	596ab	747bc	126.50c	0.14cd
	Chamran	530ab	987ab	128.84c	0.15c
	S-85-19	520ab	977ab	118.76d	0.12d
180	Dena	554ab	927ab	147.76ab	0.21ab
	Behrang	590ab	937b	151.74ab	0.22ab
	Chamran	610a	1010a	162.97a	0.24a
	S-85-19	500b	933ab	145.27ab	0.20ab
240	Dena	430bc	910ab	140.49b	0.16b
	Behrang	490b	833b	138.77b	0.17b
	Chamran	491b	857b	132.27c	0.18b
	S-85-19	466bc	817b	130.34c	0.15bc

*Means with similar letters in each column are not significantly different by Duncan's test at 5% probability level.

Assessment mean comparison result of interaction effect of treatments indicated maximum contribution of redistribution (50.50%) was noted for 180 kg.ha⁻¹ nitrogen and chamran genotype and lowest one (14.54%) belonged to nonuse of nitrogen and S-85-19 line (Table 3). Improving the capacity for supporting grain filling by stem and spike reserves is an important physiological trait and breeding target in wheat. Studies have shown that reserve remobilization could contribute to 5–20% of the final grain yield under non-stress conditions, while it might rise to 40–60% under stressful growing conditions (Blum, 1998). Golabadi *et al.* (2015) was evaluating response of wheat genotypes under drought stress

and different rate of nitrogen. Their result showed the highest values of spike and stem contribution to grain yield were obtained under drought stress while current photosynthesis was found to be the sole supplier for seed filling in normal conditions. Application of extra nitrogen fertilizer under drought stress was found to reduce the loss of seed yield in some genotypes as a result of enhanced vegetative growth, reserve accumulation, and dry matter remobilization to the seed. Pre-anthesis stored dry matter in wheat (*Triticum aestivum* L.) is important in a Mediterranean climate because grain filling greatly depends on the remobilization of pre-anthesis assimilates.

Continue table 3.

Nitrogen Fertilizer (kg.ha ⁻¹)	Genotype	Contribution of redistribution (%)	Rate of current photosynthesis (gr.m ⁻²)	Efficiency of current photosynthesis (gr.gr ⁻¹)	Contribution of current photosynthesis (%)
Control	Dena	16.69g	350.80e	0.30de	45.31de
	Behrang	18.41g	369.76e	0.30de	46.59de
	Chamran	20.25f	380.64e	0.31de	48.75de
	S-85-19	14.54h	340.35f	0.28e	40.46e
60	Dena	23.23cd	450.33d	0.34d	52.77d
	Behrang	25.20cd	430.15d	0.35d	54.80d
	Chamran	31.42c	460.83d	0.37d	58.58d
	S-85-19	20.94e	400.25d	0.32e	50.06d
120	Dena	34.84bc	510.78c	0.86c	62.16c
	Behrang	38.74bc	539.02bc	0.82cd	65.26c
	Chamran	40.01b	550.08bc	0.26f	68.99c
	S-85-19	31.81c	490.06c	0.38f	60.19c
180	Dena	46.69ab	710.51ab	0.90ab	80.31ab
	Behrang	48.38ab	727.02ab	1.92ab	82.62ab
	Chamran	50.50a	737.67a	0.95a	83.50a
	S-85-19	44.41ab	700.67ab	0.85ab	78.59ab
240	Dena	42.12ab	620.64b	0.80b	72.88b
	Behrang	44.15ab	639.35b	0.82b	73.85b
	Chamran	46.45ab	650.52b	1.84b	76.55b
	S-85-19	40.48b	600.92b	0.78bc	70.52bc

*Means with similar letters in each column are not significantly different by Duncan's test at 5% probability level.

4.6. Rate of current photosynthesis

Result of analysis of variance revealed effect of nitrogen fertilizer, genotypes and interaction effect of treatments on rate of current photosynthesis was significant at 5% probability level (Table 2). Evaluation mean comparison result of interaction effect of treatments indicated maximum rate of current photosynthesis (737.67 gr.m⁻²) was noted for 180 kg.ha⁻¹ nitrogen and chamran genotype and lowest one (340.35 gr.m⁻²) belonged to nonuse of nitrogen and S-85-19 line (Table 3). The carbon (C) necessary for grain filling in wheat comes from three sources: current assimilation; remobilization of pre-anthesis assimilates stored in the stem and other parts of the plant; and

re-translocation of assimilates stored temporarily in the stem after anthesis.

To understand the reduction in grain yield arising from post-anthesis water deficits, it is necessary to identify which of these sources of C is limiting the grain-filling process (Ercoli *et al.* 2008). On the whole, about 10% to 30% of the stem carbohydrates which are extant before and after anthesis are sent to the grain-bearing organ; this remobilization can be more than 70% in some grain crops (Setter *et al.*, 1998).

4.7. Efficiency of current photosynthesis

According result of analysis of variance effect of nitrogen fertilizer, genotypes and interaction effect of treatments on efficiency of current photo-

synthesis was significant at 5% probability level (Table 2). Assessment mean comparison result of interaction effect of treatments indicated maximum efficiency of current photosynthesis (0.95 gr.gr^{-1}) was noted for 180 kg.ha^{-1} nitrogen and chamran genotype and lowest one (0.28 gr.gr^{-1}) belonged to nonuse of nitrogen and S-85-19 line (Table 3). Decrease in seed filling duration can reduce seed filling by carbohydrates and increase of protein ratio to carbohydrate in seeds (Subedi *et al.*, 2007). Early-maturing genotypes, due to the fact that they start their productivity sooner, have a better chance for seed filling and growth. But late -maturing genotypes, due to their late productivity, and because their growth period meets heat at the end of the season, devote less time to their seed filling and growth (Abedi *et al.*, 2011).

4.8. Contribution of current photosynthesis

Result of analysis of variance revealed effect of nitrogen fertilizer, genotypes and interaction effect of treatments on contribution of current photosynthesis was significant at 5% probability level (Table 2). Evaluation mean comparison result of interaction effect of treatments indicated maximum contribution of current photosynthesis (78.59%) was noted for 180 kg.ha^{-1} nitrogen and chamran genotype and lowest one (40.46%) belonged to nonuse of nitrogen and S-85-19 line (Table 3). Ehdai *et al.* (2006) noted that wheat crops in dry land areas may depend more on stem reserves for grain filling than on current photosynthesis. In high

levels of nitrogen consumption, due to productivity of more leaves and their persistence, because of having high amount of current photosynthesis, have decrease in amount of redistribution (Mohammadi *et al.*, 2015).

5. CONCLUSION

Finally according result of current study application 180 kg.ha^{-1} nitrogen and chamran genotype had the highest amount of studied traits and it can be advice to producers in studied region.

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FOOTNOTES

CONFLICT OF INTEREST: Author declared no conflict of interest.

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