Tropical Agricultural Research Vol. 12: 22-32 (2000)

Physiological Responses of Maize (*Zea mays* L.) to the Interactive Effects of Nitrogen Fertilizer and Water Regimes

S. Satchithanantham and D.C. Bandara[1]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT. *Interactive effects of nitrogen and water on physiological processes associated with yield determination of maize have received little attention. This study examined how nitrogen and water interactive effects influenced physiological parameters and quantified the yield response of maize in intermediate dry zone of Sri Lanka. Variety* Ruwan *was grown in the University of Peradeniya Research Station, Dodangolla, at 3 nitrogen levels under rainfed and irrigated conditions in a split plot design. The canopy formation, leaf chlorophyll contents, leaf nitrogen concentration, photosynthesis rate and yield were studied in the field experiment.*

Increased nitrogen fertilizer application positively influenced all above parameters under supplementary irrigation while water stress markedly affected these parameters after the silking stage. The leaf area index was lowest in the no nitrogen, water stressed treatments but irrigation improved it even without nitrogen application. Lack of nitrogen application under water stressed condition caused 49%, 36% and 52% reductions in the peak values of leaf chlorophyll, leaf nitrogen contents and leaf photosynthesis from that of high nitrogen treated irrigated plants. Rapid decline in above parameters and leaf area index caused reduction in total dry weight after the silking stage in nitrogen applied water stressed treatments and it was reflected in the grain yield. Lack of nitrogen under water stressed condition caused 77% reduction in grain yield from that of high nitrogen treated irrigated plants. The interactive effect of nitrogen and water was significant on leaf nitrogen content, photosynthesis, leaf area index and total dry weight during grain-filling stage while it was significant on leaf chlorophyll throughout the season.

INTRODUCTION

Among the world's cereal crops, maize (*Zea mays* L.) ranks 2nd only to wheat in production. Maize has been put to a wider range of uses than any other cereal as human food, a feed grain, a fodder, and for hundreds of industrial purposes. Maize is very popular for both fresh cobs and dry grains in Sri Lanka. The average annual consumption of maize in Sri Lanka is 95,000 MT.

The potential average yield of var. *Ruwan* is 4365 kg ha^{-1} (Muthukuda Arachchi, 1990). The local production however does not reach even a half of the requirement even though maize is a C4 plant and can be grown well under the tropical conditions of Sri

1 Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

Lanka. This low yield is attributed to various reasons such as, moisture stress, poor fertility in soil, poor crop management practices and pest and diseases.

The two physical factors most responsible for limiting maize production in developing countries are drought stress and soil infertility. In Sri Lanka maize is grown mainly as a rainfed crop during *Maha*. However, it can also be grown during *Yala* only under irrigation. Moisture stress has been a major cause of yield reduction in maize as it is grown in the dry zone under rainfed conditions. Drought affects maize grain yield to some extent at almost all growth stages (Heisey and Edmeades, 1998).

Nitrogen (N) nutrient is essential to maize growth and development. A strong correlation has been demonstrated between net photosynthetic rate and leaf nitrogen (Sinclair and Horie, 1989). Plants that are deficient in nitrogen will have lower photosynthetic rates and, as a result, will accumulate less dry matter and produce lower yields (Dwyer *et al.*, 1995).

Interactive effects of nitrogen and water regimes in maize under Sri Lankan condition should be studied to recommend the nitrogen level in different water regimes for optimum production. Many studies have focused on either water or nitrogen deficits but essentially ignored the complex interaction between water and nitrogen that have an impact on leaf development and senescence (Wolfe *et al.*, 1988). These interactions are particularly significant in a field situation when stress intensity gradually increases during the growing seasons. Previous research on the interaction of water and N stress have suggested that N stress alters several morphological and physiological characters, which might be responsible for differential responses to water stress. Therefore, it can be hypothesized that the physiological processes of maize are sensitive to water and N stresses and their interaction, and that final grain yield would respond positively to increased application of nitrogen fertilizer and irrigation. Hence, the objectives of the present study were to examine the way in which N and water interactive effects influence the physiological parameters and to quantify the yield response of maize under a selected Sri Lankan growing condition.

MATERIALS AND METHODS

The experiments were conducted at the University Experimental Station, Dodangolla, during 1999 *Yala*. The selected site was situated in the mid country intermediate zone of Sri Lanka (Pannabokke, 1996). The annual average rainfall was 1400 mm having a distinctly bi-modal distribution. The mean temperature of the experimental period was 29.14^{0}C. Maize (*Zea mays* L.), variety *Ruwan*, which is a 120 days crop was used. The soil on which the experiments were carried out was sandy clay loam belonging to the great group Reddish Brown Latosolic soils with pH of 6.5. The initial total soil nitrogen level was 0.1%.

Three levels of N treatments N_0, N_1 and N_2 were made by 0, 37.5 and 75 kg ha^{-1} of urea for the basal dressing and 0, 75 and 150 kg ha^{-1} of urea for the top-dressing (total urea 0, 112.5 and 225 kg ha^{-1}) respectively (the recommendation of Department of Agriculture is 150 kg ha^{-1}). These treatments were tested in both irrigated (I1) and rainfed

(I0) conditions. The combinations of nitrogen and irrigation treatments were N0I0, N1I0, N2I0, N0I1, N1I1 and N2I1. Irrigation was done once in 4 days until 2 weeks before harvesting as recommended by the Department of Agriculture (Anonymous, 1990). During each irrigation the relevant plots were brought to field capacity. A split plot design with irrigation treatment as the main factor and nitrogen treatments as split plot factor was used with three replicates. The individual plot size was 16 square meter (4 m × 4 m) with two meters of space around each plot to overcome seepage of water to nearby plots. The planting density used was 55,000 plants ha^{-1}.

Other than urea the land was fertilized with triple super phosphate and muriate of potash at the rate of 150 kg ha^{-1} and 50 kg ha^{-1} respectively according to the recommendation of the Department of Agriculture. Apart from the different treatments all crops were managed similarly. The total crop duration of maize was divided into 4 stages as knee-high, vegetative, flowering and grain filling stages. The knee-high stage was the period from planting to the time it reaches knee-high height (0–28 DAP). The vegetative period was between knee-high and the observation of visible tasselling (28–51 DAP), while flowering stage was between observation of tasselling and 5 days after 75% silking (51–65 DAP). The grain-filling stage was between five days after 75% silking and harvests (65–105 DAP) (Seneviratne and Appadurai, 1966).

At 30, 45, 58, 72 and 86 days the following parameters were measured; leaf area index (LAI) by a portable leaf area meter (Li-3000, Li-CO-R, Inc.), total dry weight (TDW in g) by drying various plant parts at 80°C and weighing, leaf chlorophyll ($\mu g/cm^2$) was calculated from light absorbency of acetone extract of chlorophyll, total leaf N (%) by Kjeldahl apparatus and photosynthesis ($\mu mol/m^2/s$) by a portable photosynthesis system (Li- 6400, Li-CO-R, Inc.). The above parameters other than LAI and TDW were measured in the most recently expanded leaf. Photosynthesis measurements were taken from five plants in each plot between 10.00 a.m to 1.00 p.m on every sampling date. The sampling dates coincided with different growth stages. Total dry weight and leaf area index was measured by destructive sampling of 5 randomly selected plants. Yield was measured by harvesting a pre-designated 1 m^2 area from the middle of the plot at 101 DAP. Analysis of variance (ANOVA) of the measured data was carried out on every sample separately using the SAS statistical package.

RESULTS AND DISCUSSION

General observations

The total rainfall during the experimental period was 269.5 mm. Of this 207.1 mm rainfall was received by the crop within 23 days after planting (knee-high stage). The rainfall received by the crop during vegetative, flowering and grain filling stages was 62.4 mm. The pan evaporation during these 3 stages was 213.1 mm. Therefore, the experimental crop experienced adequate water stress. N and water stressed plants showed low heights and pale green colour leaves compared to other plants. Among irrigated plants the N1 treatment did not show much difference in height and leaf colour compared to the N2 treatment plants.

Leaf area index (LAI) and total dry weight (TDW)

Application of nitrogen fertilizer significantly increased LAI at all sampling dates (Table 1). LAI of medium and high nitrogen plants under irrigation (I1N1 and I1N2) attained their maximum at the flowering stage (at 58 DAP) and thereafter during the grain-filling period it decreased but was maintained relatively high (Fig. 1a). The I1N2 had the highest LAI at almost all-sampling dates. Peak LAI of the medium and high nitrogen plants that were under rainfed condition (I0N1 and I0N2) reduced approximately by 31% and 35% respectively, compared to the corresponding irrigated plants at 58 DAP and thereafter decreased. At 86 DAP it became less than half the value of their respective peak LAI. The rapid reduction in LAI after the silking stage may be due to high transpiration demand and a high reproductive sink demand for nitrogen (Wolfe *et al.*, 1988).

Table 1. Effect of nitrogen fertilizer and water regimes on different physiological parameters and yield.

Source	LAI	TDW	Lchl	Lnit	LPhs	Yld
Water (W)	***	***	***	***	***	***
Nitrogen (N)	***	***	***	***	***	***
W*N	**	***	***	***	**	***
CV%	26.05	15.03	9.25	12.92	6.58	7.81

, * - Significant at $p<0.01$, $p<0.001$ respectively; LAI - Leaf area index
TDW - Total dry weight (g/m^2); Lchl - Leaf chlorophyll ($\mu g/cm^2$)
Lnit - Leaf nitrogen (%); Lphs - Leaf photosynthesis ($\mu mol/m^2/s$)
Yld - Yield ($kg\ ha^{-1}$)

As maize is a determinate crop it produced its leaves only during vegetative stage. Therefore, to achieve maximum expansion of the initiated leaves irrigation is necessary during the vegetative stage. Plants without nitrogen application under rainfed condition (I0N0) had the lowest LAI while the irrigated plants (I1N0) had twice the value than that of I0N0. Irrigation had significant effect on LAI at all sampling dates except 45 DAP while it had significant effect on TDW at 58, 72 and 86 DAP. The pattern of LAI in response to different treatment combinations almost coincides with the pattern of response in TDW (Fig. 1b).

This result confirmed previous observations (Novoa and Loomis, 1981; Muchow, 1988; Vos and Van der Putten, 1998) showing that nitrogen had a marked influence on leaf area development and the sensitivity of leaf expansion to water deficit (Turner and Begg, 1981). The effect of nitrogen on both leaf cell number and size would be the reason for increasing leaf area. The maintenance of green leaves for a long period even in grain-filling

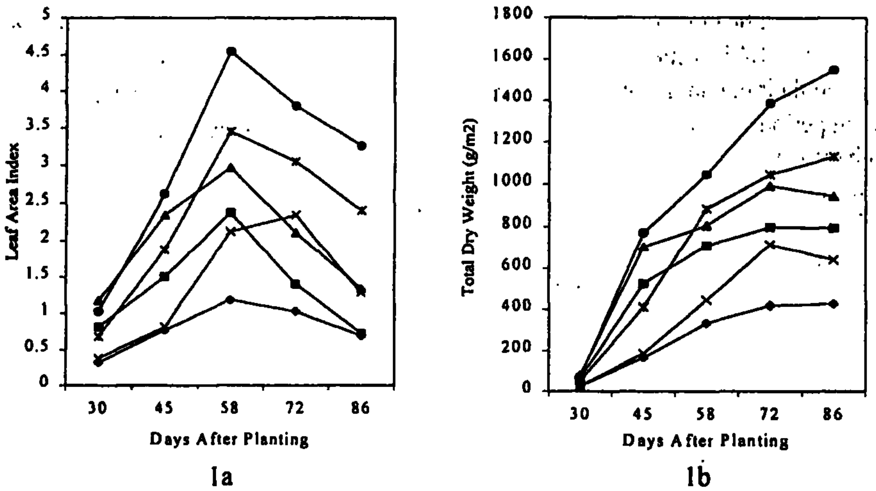
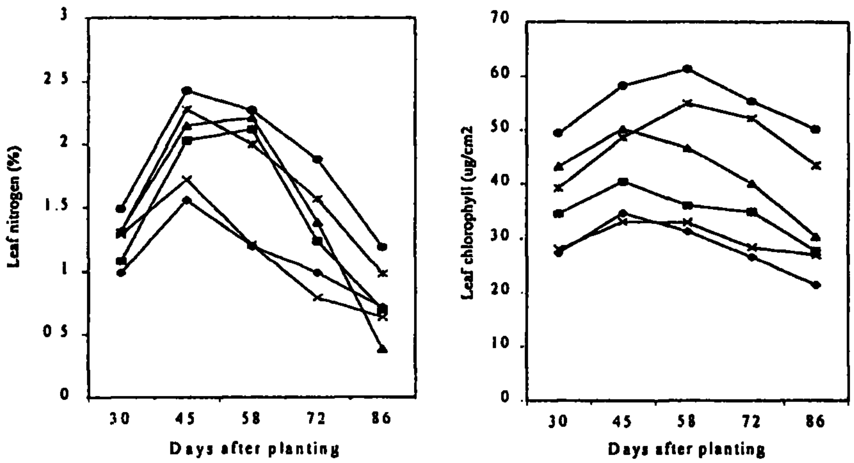


Fig. 1. (a) Seasonal variation of leaf area index and (b) Total dry weight in response to nitrogen fertilizer and water regime.
[Note: ◆-I0N0, ■-I0N1, ▲-I0N2, x-I1N0, ✱-I1N1, ●-I1N2. LSD on any date of sampling between different N treatments at the same irrigation treatment are 0.57 for LAI and 90.50 for TDW and LSD between irrigation treatments at the same nitrogen levels are 0.47 for LAI and 73.90 for TDW].

stage would have enabled the crop to intercept radiation for a long period and increased biomass accumulation through photosynthesis in I1N2 treatment. The reason for decrease in biomass production in nitrogen stressed plants could be either a reduction in the amount of radiation intercepted by the canopy or from a decrease in the efficiency with which the intercepted radiation is used to produce dry matter or a combination of both these factors. Muchow (1988) stated that Radiation Use Efficiency (RUE) of maize was more responsive to N supply than was radiation interception and RUE increased with higher rates of applied nitrogen. Only in the late grain filling stage was there an interaction between nitrogen and water on LAI and TDW.

Leaf chlorophyll and leaf nitrogen

Nitrogen fertilizer application significantly increased leaf nitrogen and leaf chlorophyll contents at all sampling dates (Table 1 and Fig. 2a and 2b). Irrigation had significantly increased the above parameters at all sampling dates except at 58 DAP considering leaf nitrogen content. The I1N2 treatment maintained high leaf nitrogen and leaf chlorophyll contents throughout the growing season. Water stress highly affected leaf nitrogen and leaf chlorophyll contents after silking stage when severe water stress gradually developed in the field (Fig. 2). The decline of leaf chlorophyll preceded the decline of leaf nitrogen in I0N1 and I0N2 treatments. Wolfe *et al.* (1988) also reported this observation. This observation was evidenced by a significant effect of irrigation on leaf chlorophyll but not on leaf nitrogen at 58 DAP. Water stress caused 11–18% reduction in leaf nitrogen in the vegetative stage possibly due to lack of sink demand for nitrogen.



2a 2b

Fig. 2. (a) Seasonal variation of leaf nitrogen (N) and (b) Leaf chlorophyll (LC) in response to nitrogen fertilizer and water regime.

[Note: ◆-I0N0, ■-I0N1, ▲-I0N2, x-I1N0, ✱-I1N1, ●-I1N2. Least significant difference (LSD) on any date of sampling between different nitrogen treatments at the same irrigation treatment are 0.27 for leaf N, 5.40 for LC and LSD between irrigation treatments at the same nitrogen levels are 0.22 for leaf N and 4.41 for LC].

During reproductive growth of maize, grain nitrogen is supplied from vegetative tissue as well as from concurrent nitrogen uptake (Pan *et al.*, 1986; Ta and Weiland, 1992). Although absorption of total nitrogen from soil continue during the grain filling period, 60 to 85% of the total N present at anthesis remobilise to the ear (Ta and Weiland, 1992). This explains the gradual decrease of leaf nitrogen in non-stressed maize plants in our study. A reduction in the uptake of N induced by a water deficit has been well-documented (Begg and Turner, 1981). In our experiment the interaction between N and water on leaf N was significant only after 72 DAP when the water stress became more severe during grain filling stage. Nutrient levels in the field are usually highest near the surface of soil.

Although water stressed high N plants can effectively extract water from the deeper soil profile, the relative lack of nutrients in the sub soil and the unavailability of nutrients in the dry surface soil would cause water stress induced reduction in N uptake in I0N1 and I0N2 treatments. The resulting low N availability enhanced N mobilization from leaves and this would cause low leaf N levels in water stressed maize plants. The low leaf nitrogen level caused low photosynthesis rate in I0N1 and I0N2 treatments due to the high correlation between leaf nitrogen and photosynthesis in maize (Sinclair and Horie, 1989).

Leaf photosynthesis

Nitrogen and water effects on photosynthesis were both significant and positive at all sampling dates. The interactive effect between N and water on photosynthesis was significant at 72 DAP and 86 DAP (Table 1 and 2). The high leaf nitrogen and leaf chlorophyll contents can be correlated to the high photosynthesis rate in I1N2 treatment. The I0N0 treatment caused approximately 38% reduction in photosynthesis from that of I1N2 treatment during vegetative stage (Fig. 3a). When leaf nitrogen and leaf chlorophyll contents rapidly decreased after silking stage, photosynthesis was also affected by water stress in I0N1 and I0N2 treatments.

Table 2. Leaf photosynthesis as affected by water and nitrogen treatments.

Treatment	Photosynthesis (µmol/m²/s)				
	Days After Planting				
	30	45	58	72	86
I0N0	27.34	26.03	22.48	20.19	16.62
I0N1	33.27	34.49	37.69	31.08	25.62
I0N2	34.93	36.59	41.33	32.96	24.25
I1N0	28.86	31.21	28.08	24.34	18.67
I1N1	35.46	38.91	41.39	37.52	26.42
I1N2	37.63	41.96	46.65	43.05	31.44
LSD (0.05)	2.08	2.59	1.91	2.01	2.60

As nitrogen is a component of key photosynthetic enzyme (RuBPcase) lack of nitrogen caused reduction in the amount of RuBPcase (Osaki *et al.*, 1993) and this would lead to low photosynthesis rate. The high sink demand for leaf nitrogen and water stress induced nitrogen deficiency in I0N2 treatment would cause remobilisation of leaf nitrogen from leaf to grain. This can explain the rapid decline in leaf nitrogen and consequently rapid decrease in photosynthesis. The reduction of photosynthetic capacity by the mobilization of nitrogen from leaf to grain during reproductive development was also stated by Muchow and Sinclair (1994). There is much literature to prove great reduction in grain yield by water stress during flowering and grain filling stages (Heisey and Edmeades, 1998). In our study also the increased water stress in the field coincided with the flowering and grain filling stages and this can explain the low yield gain in water stressed treatments (Fig. 3b).

High N level is accompanied by increases in both mesophyll and stomatal conductance in C4 plants (Bolton and Brown, 1980). It is generally accepted that the initial reduction in photosynthesis due to an increase of water deficit arises from changes in stomatal conductance. Water stress decreases the rate of net photosynthesis per unit leaf area (Turner and Begg, 1981).

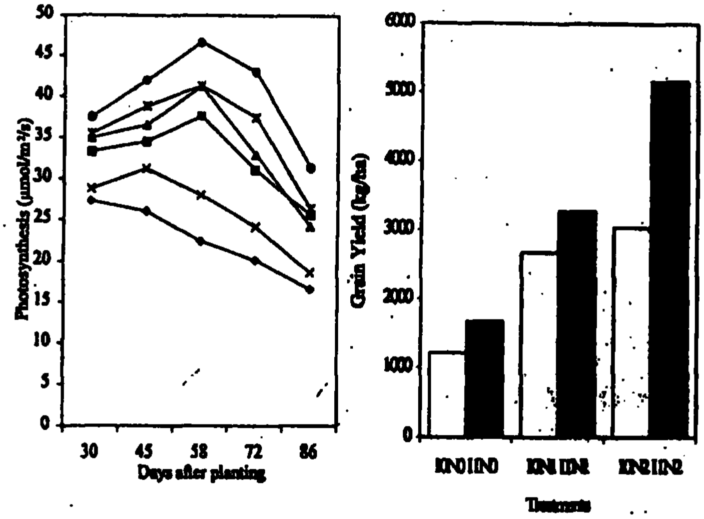


Fig. 3. (a) Seasonal variation of leaf photosynthesis and (b) Grain yield in response to nitrogen fertilizer and water regime.
[Note: ◆-I0N0, ■-I0N1, ▲-I0N2, x-I1N0, ✱-I1N1, ●-I1N2].

Grain yield

N and irrigation effects on grain yield were both significant and positive. The interactive effect of N and water on grain yields was also significant (Table 1 and 3). The treatment I1N2 had the highest yield (5164 kg ha^{-1}). The yield of I1N1 treatment was approximately the same as that of I0N2 with a small difference. The treatment I0N1 had lower yield than that of I1N1 treatment (Fig. 3b). Water stress highly affected the grain yield when high N was applied.

The treatments I1N0, I0N2 and I0N0 showed grain yield reductions of 68%, 41% and 77% respectively, from that of I1N2 treatment. Meanwhile, the treatments I1N0, I0N1 and I0N0 showed reductions of 49%, 18% and 63% respectively, from that of I1N1 treatment.

The demand for carbon and N by the grain can be met by current carbon assimilation and N uptake during grain filling and by the mobilization of pre-anthesis assimilate and nitrogen. Since most of the carbohydrate stored in the grain is derived from post-anthesis assimilation in cereals, the photosynthetic activity during grain filling are critical determinants of grain yield (Muchow, 1988). The low photosynthesis rate of I0N2 may be caused by N deficiency due to water stress and low demand for nitrogen due to reduced growth caused by water stress during grain filling stage. This explained the reduced grain yield of I0N2 treatment. In addition, to the above reasons the low grain yield of I0N1 treatment may also be due to inadequate nitrogen fertilizer application. Water stress in the field would reduce the diffusion and mass flow of nitrate ion to the root system resulting in low nitrate uptake. Water stress reduce nitrate reductase activity and

Table 3. **Grain yield as affected by water and nitrogen treatments.**

Treatment	Yield (kg ha^{-1})
I0N0	1215.10
I0N1	2679.70
I0N2	3042.60
I1N0	1679.00
I1N1	3271.00
I1N2	5163.60
LSD (0.05)	567.52

as a result protein synthesis becomes affected. This would cause reduced growth of the water-stressed plants. Prolonged accumulation of dry matter and N by above ground plant parts of maize during grain filling has been reported as important characteristics associated with high yields (Swank *et al.*, 1982; Moll *et al.*, 1994).

CONCLUSIONS

This study showed that increased application of N fertilizer significantly increased leaf N and leaf chlorophyll content through which photosynthesis capacity was also increased when the plants were given supplementary irrigation. When these plants were subjected to water stress the above parameters rapidly declined and as a result photosynthetic capacity also decreased. The lack of N caused leaf chlorophyll and leaf N contents to be lower in both water stressed and irrigated plants. However, irrigation increased leaf area index and photosynthetic capacity and thus total biomass accumulation. The significance of interactive water and N effects on yield was supported by physiological measurements of young leaf. The leaf chlorophyll content, leaf N content and photosynthetic capacity of the young leaves were affected in the N applied water stressed plants in the latter stages of the life cycle of the plant. This lead to the yield reduction in high N applied water stressed plants compared to the medium N applied irrigated plants.

The recommended level of nitrogen by the Department of Agriculture (150 kg ha^{-1} urea) gives yield of 1500 to 1700 kg ha^{-1} under rainfed condition and 2500 to 4000 kg ha^{-1} under irrigated condition (Anonymous, 1997). The present study showed that nitrogen increment of 75 kg ha^{-1} urea more than the recommended urea level gives yield increment of 103% under rainfed condition and 107% under irrigated condition. However, irrigated treatment gave 69.7% of yield increment compared to the rainfed treatment. Therefore, it can be concluded that application of increased N fertilizer will be economical when plants are given supplementary irrigation. This recommendation was based on the results of a one season experiment conducted at an intermediate dry zone area. Further studies of nitrogen and water interactive effects on physiological processes of maize in different locations in Sri Lanka is necessary for a general recommendation of increased nitrogen fertilizer application at different water regimes.

ACKNOWLEDGMENTS

This work was funded by a research grant from the Postgraduate Institute of Agriculture. The assistance from staff of the experimental station, Dodangolla, Department of Agricultural Biology and Department of Crop Science are gratefully acknowledged.

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